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Computational Image Analysis Reveals Intrinsic Multigenerational Differences between Anterior and Posterior Cerebral Cortex Neural Progenitor Cells

Mark R. Winter,[1] Mo Liu,[3] David Monteleone,[1] Justin Melunis,[2] Uri Hershberg,[2] Susan K. Goderie,[3] Sally Temple,[3,*] and Andrew R. Cohen[1,*]

[1]Department of Electrical and Computer Engineering, Drexel University, Philadelphia, PA 19104, USA
[2]Department of Biomedical Engineering and Science, Drexel University, Philadelphia, PA 19104, USA
[3]Neural Stem Cell Institute, Rensselaer, NY 12144, USA
*Correspondence: sallytemple@neuralsci.org (S.T.), acohen@coe.drexel.edu (A.R.C.)
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SUMMARY

Time-lapse microscopy can capture patterns of development through multiple divisions for an entire clone of proliferating cells. Images are taken every few minutes over many days, generating data too vast to process completely by hand. Computational analysis of this data can benefit from occasional human guidance. Here we combine improved automated algorithms with minimized human validation to produce fully corrected segmentation, tracking, and lineaging results with dramatic reduction in effort. A web-based viewer provides access to data and results. The improved approach allows efficient analysis of large numbers of clones. Using this method, we studied populations of progenitor cells derived from the anterior and posterior embryonic mouse cerebral cortex, each growing in a standardized culture environment. Progenitors from the anterior cortex were smaller, less motile, and produced smaller clones compared to those from the posterior cortex, demonstrating cell-intrinsic differences that may contribute to the areal organization of the cerebral cortex.

INTRODUCTION

Time-lapse microscopy enables the patterns of development, cellular motion, and morphology to be observed and captured for clones of proliferating cells. Phase contrast microscopy allows image capture at a temporal resolution sufficient for accurate tracking through multiple rounds of cell division in a label-free manner. By integrating appropriate incubation, live cell development can be imaged over a period of days or even weeks. An experiment can produce 350 gigabyte (GB) of image data and there is a pressing need for efficient analytical computational tools.

In general, humans are better able to correctly identify and track cells than the best available software, but manual tracking is prohibitively slow. In order to efficiently analyze time-lapse phase image sequences of proliferating cells, the best current approach is to combine human visual capabilities with automated image analysis algorithms.

Human validation is essential to correct errors produced by the automated programs, which fall into three classes: segmentation, tracking, and lineaging errors. Segmentation identifies individual cells in each image. A segmentation error has occurred if a cell is not correctly detected. Tracking is the process by which objects are followed from one frame to another. Tracking errors occur when segmentation results identifying different cells are associated on the same track. Lineaging errors occur when the parent-daughter relationships are incorrectly identified. Our algorithms allow some segmentation errors, such as when a cell is obscured for a single frame, but all tracking and lineaging errors must be corrected. Human validation corrects these errors and the goal is to minimize the user corrections required.

The clones used in this study were derived from neural progenitor cells (NPCs) extracted from the embryonic mouse cerebral cortex. NPCs include neural stem cells and more restricted progenitor cells. The cortex performs numerous functions, integrating sensory information, thought, and memory with appropriate behavioral responses. Different cortical functions are achieved through areal specializations. For example, the visual cortex is concerned with processing information derived from the retina, while the motor cortex drives movement via subcortical connections to the spinal cord. The visual cortex arises in the posterior region of the embryonic telencephalon, and the motor cortex arises from the anterior region. How these two distinct areas develop differently from each other is an important question in developmental neurobiology. It is possible that the anterior and posterior NPCs are intrinsically similar and rely on the presence of growth factor gradients (O'Leary et al., 2007) to direct their output. Alternatively, the growth factor gradients might instill cell-intrinsic changes in the NPCs to alter their behavior. In order to discern these two possibilities, we need to study the growth of anterior and posterior NPCs exposed to the same environment, which can only be done ex vivo. The hypothesis we tested is that anterior and posterior cortical NPCs are intrinsically different, reflected in different lineage outputs and behaviors when cultured in a standardized environment.

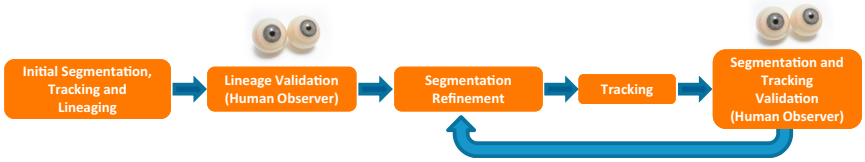

Figure 1. Overview of Approach
Starting with an initial segmentation, cells are tracked through the image data and a lineage is obtained. The parent-daughter relationships in the lineage are validated by the human observer. The validated lineage is then used to refine the segmentation and tracking under supervision. This refine and then validate process is repeated for each image, achieving a significant reduction in the segmentation error rate.

RESULTS

E12.5 mouse anterior or posterior cortical NPCs were plated in a 24 well plate at clonal density in serum-free culture medium, with images captured every 5 min for over 4 days. Image data gathered in three separate experiments was initially segmented, tracked, and lineaged, according to the process outlined in Figure 1. These initial segmentation and tracking algorithms have been applied in a number of recent applications (Chenouard et al., 2014; Cohen et al., 2009, 2010; Mankowski et al., 2014; Winter et al., 2011, 2012). We developed a new segmentation algorithm that uses lineage information to automatically improve segmentation and tracking accuracy in a step referred to as "post-lineage refinement". The post-lineage refinement uses the parent-daughter information that is challenging for current machine vision approaches (Seungil et al., 2011), but relatively fast and easy for a human to identify. The segmentation and tracking results were then automatically refined from the corrected lineage information with human observers correcting any remaining segmentation and tracking errors interactively. All of the validation was done using a program called Lineage Editing and Validation (LEVER) (Winter et al., 2011). LEVER allows a user to visualize the lineage tree together with the segmentation and tracking results. The results are color coded in order to make errors as easy to identify as possible. Manual edits and the automatic corrections are logged and counted to determine the error rates of the different algorithms. All of the software and algorithms are available free and open source as detailed below.

Figure 2 shows a montage of all 160 lineage trees, a total of 10,644 cells and 1,585,104 segmentations. Movie S1 shows a sample movie for a posterior clone with segmentation and tracking overlaying the image data in the left panel and the lineage tree in the right panel. Our web-based visualization program CloneView provides an interactive way to explore the data and results. Figure 3 shows a screen shot of the CloneView program with a summary listing the clones in one window and one image frame with segmentation and tracking results overlaid in the other window. All of the image data, together with all segmentation and tracking results, are available through our web-based tool called CloneView. CloneView runs on any computer that supports a modern web browser with no software to download. CloneView is available at http://n2t.net/ark:/87918/d91591.

The initial segmentation algorithm error rate of 8.1% represents all the segmentation errors including both the automatic corrections generated by the post-lineage refinement (6.4%) and the user-provided manual corrections (1.7%). This represents a 79% reduction in segmentation error rate compared to the initial segmentation. This initial segmentation incorporates our previous development of stem cell segmentation algorithms (Mankowski et al., 2014; Wait et al., 2014; Winter et al., 2011). The tracking error rate was 1%. The total error rate, calculated from the number of edit operations required to fully correct the segmentation, tracking, and lineaging errors, was 1.3%. Once validated, we can extract features such as cell lifespan, location, and size, enabling quantification of the cell-cycle time, motion, and morphology for individual cells, across clones and broken down by generation. The analysis of this feature data reveals significant differences in the patterns of development between anterior and posterior cerebral cortical NPCs.

The Lineage Tree Is Used to Refine the Underlying Segmentation

Of all the tasks required for this analysis, segmentation, or delineation of individual cells in each image frame is the most challenging and error prone. Even human observers can find it difficult to establish the correct number of cells in a close group from a single image. When the number of cells has been correctly established, clustering models that incorporate morphological characteristics of the cells, together with temporal information from the tracking, reliably separate the foreground pixels into individual cells.

We begin with an initial segmentation algorithm originally developed for phase contrast images of retinal stem cells (Cohen et al., 2010) and applied previously to neural stem cells (Winter et al., 2012). Modified versions of this segmentation algorithm have been applied to oligodendrocyte precursors (Cohen et al., 2010) and hematopoietic