Bioinformatics Advance Access published May 18, 2013

BIOINFORMATICS APPLICATIONS NOTE 2013, pages 1–2
doi:10.1093/bioinformatics/btt194

Systems biology

Advance Access publication April 23, 2013

CellAging: a tool to study segregation and partitioning in division in cell lineages of *Escherichia coli*

Antti Häkkinen[1], Anantha-Barathi Muthukrishnan[1], Andre Mora[2], Jose M. Fonseca[2] and Andre S. Ribeiro[1,*]

[1]Department of Signal Processing, Tampere University of Technology, 33101 Tampere, Finland and [2]Department of Electrotechnical Engineering, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Lisboa, Portugal

Associate Editor: Martin Bishop

ABSTRACT

Motivation: Cell division in *Escherichia coli* is morphologically symmetric. However, as unwanted protein aggregates are segregated to the cell poles and, after divisions, accumulate at older poles, generate asymmetries in sister cells' vitality. Novel single-molecule detection techniques allow observing aging-related processes *in vivo*, over multiple generations, informing on the underlying mechanisms.

Results: CellAging is a tool to automatically extract information on polar segregation and partitioning in division of aggregates in *E.coli*, and on cellular vitality. From time-lapse, parallel brightfield and fluorescence microscopy images, it performs cell segmentation, alignment of brightfield and fluorescence images, lineage construction and pole age determination, and it computes aging-related features. We exemplify its use by analyzing spatial distributions of fluorescent protein aggregates from images of cells across generations.

Availability: CellAging, instructions and an example are available at http://www.cs.tut.fi/%7esanchesr/cellaging/.

Contact: andre.ribeiro@tut.fi

Supplementary information: Supplementary data are available at *Bioinformatics* online.

Received on March 21, 2013; revised on April 12, 2013; accepted on April 19, 2013

1 INTRODUCTION

The division process of *Escherichia coli* is morphologically symmetric. In favorable environments, the process can be sustained indefinitely. However, to be sustainable, *E.coli* needs to deal with the accumulation of unwanted substances and degradation of internal structures. Recent evidence suggests that in growing populations of isogenic cells, there is a continuous emergence of functional asymmetries in the vitality of sister cells, which are associated to the accumulation of unwanted protein aggregates at the cell poles (Lindner *et al.*, 2008). Namely, *E.coli* appears to use a bet-hedging strategy during cell division (Lloyd-Price *et al.*, 2012), in that the daughter cell inheriting the older pole of mother cell (and the unwanted aggregates within) exhibits slower growth and division rates, as well as an increased incidence of death. A recent study observed this process, one event at a time, showing that aggregates of RNA molecules tagged with MS2–green fluorescent protein, produced in the midcell region, are quickly transported to the poles, where they remain thereafter (Lloyd-Price *et al.*, 2012). Also, the choice of pole was biased in that, on average, more aggregates moved toward the older pole.

Little is known about the mechanisms underlying the process of segregation and partitioning in division of unwanted aggregates. In Lindner *et al.* (2008), it was hypothesized that the limited diffusion of the aggregates, along with cell division and nucleoid occlusion, may suffice to explain the observations. Other studies suggest that active transport mechanisms may be involved (Rokney *et al.*, 2009). To study these processes and underlying sources, one needs to observe them *in vivo*, using single-molecule visualization techniques, in growing isogenic cell populations over several generations.

Information extraction from time-lapse microscope images of growing cell populations is fastidious and technically difficult. It requires large data sets, involves multiple steps, such as cell segmentation and tracking, and requires identifying, e.g. cell lineages. Also, it is necessary to extract spatiotemporal distributions of fluorescent proteins and quantify their partitioning in divisions. There are tools available to perform some of these steps, e.g. of cell segmentation (Carpenter *et al.*, 2006). However, some steps, e.g. cell tracking, remain problematic. Also, no tool performs all the steps needed to, from the images, outputting information on segregation to the cell poles and partitioning on unwanted aggregates in cell division.

We present CellAging, a tool that performs segmentation of *E.coli* cells in brightfield images, aligns the segmentation results with fluorescence images of the same cells and establishes temporal relationships between the cells. Additionally, CellAging outputs both aging-related statistics of choice and the information on the temporal evolution of the fluorescence in each cell at the pixel level in the established hierarchy of cells. The program is operated via a graphical user interface. Aside from studies of cellular aging, it allows studying the *in vivo* dynamics of the spatial distribution and the partitioning in division of any fluorescence-tagged intracellular elements. An example application is provided in supplement, along with a detailed description of the methods used by CellAging.

2 METHODS

From a set of temporal images obtained by brightfield microscopy, CellAging first automatically segments the cells in each frame. The

*To whom correspondence should be addressed.

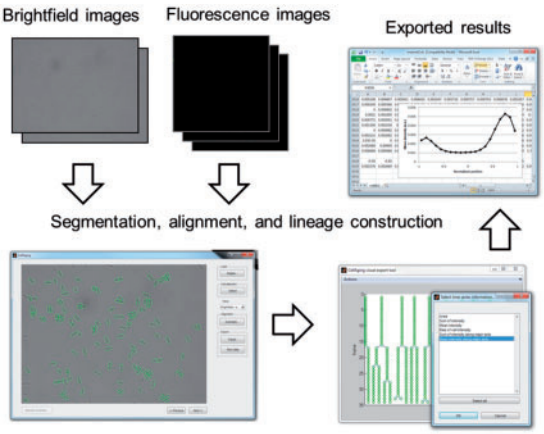

Fig. 1. Example of CellAging in use. Images (upper left) are provided to CellAging (bottom left), which analyze the time series (bottom right). Exported results, such as spatial distribution of fluorescence inside the cell, can be computed plotted using, e.g. Microsoft Excel (upper right)

segmentation is seeded by Gradient Path Labeling (Mora *et al*., 2011), and it relies on classifiers for merging and discarding segments. The classifiers were constructed using the Classification and Regression Trees algorithm (Breiman *et al*., 1984), and they were trained by an expert (Queimadelas *et al*., 2012). Inter-frame information is used to enhance the quality of the segmentation. After the automated segmentation, the results can be manually corrected. As a side note, we observed that CellAging, because of thorough training, and given consistency between images from multiple time series, consistently outperformed currently available state-of-the-art tools for the set of images that we produced in laboratory Carpenter *et al*. (2006); Young *et al*. (2011).

CellAging then automatically aligns the fluorescence images with the segmentation results. Then, it finds cell divisions and establishes the correspondence of the same cells between consecutive frames (Supplementary Material). We observed that the number of errors in this step is negligible in the images used in our case study ($<1\%$). The result can be visualized by a graphical tool, from which features can be selected and exported.

There are three categories of features (see manual). Features relative to time series of individual cells include their area, total fluorescence intensity, bias in intensity of old and new poles and the distribution of fluorescence along the major axis of the cell. These features are extracted as a function of time. Features relative to cell division include the area and the total fluorescence of the mother cell, when last observed, its division time and the bias in daughter cell areas or intensities after their appearance. Features relative to cell populations include mean cell area and mean fluorescence intensity during their lifetime, the bias in fluorescence intensity in the frame where the cells were first or last observed and the division time of each cell. From the exported results, one can either extract the full distribution of values or calculate statistics, such as mean and standard deviation.

The set of features calculated is intended to suffice to study segregation and partitioning in division of unwanted protein aggregates and consequence aging. However, to ensure applicability of CellAging for other aims, one can extract the information on the temporal evolution of each cell at a pixel level, along with the information on the relationship between the cells and on the orientation of the poles of cells born during the measurement period.

In supplement, we exemplify the usage of CellAging (exemplified in Fig. 1) by analyzing images of *E.coli* K-12 strain SX4 cells harboring the tsr-venus gene under the control of lac promoter (Yu *et al*., 2006), a gift from Sunny Xie, Harvard University, USA. The supplement also features a performance analysis of the segmentation, a description of the alignment of brightfield and confocal images and a description of the construction of lineages. Finally, we describe the strain, induction of the target gene and microscopy imaging.

3 DISCUSSION

CellAging is the first tool made available to assist studies of cellular aging using the model organism *E.coli*. We expect it to be valuable in future studies, as it allows automated high-throughput analysis of large amounts of data and unbiased comparisons between independent measurements in identical and different conditions.

CellAging provides information on the temporal evolution of many variables associated to cellular aging at three levels of detail: time series, cell divisions and population statistics. Additionally, it can also extract information at a lower level of detail, namely, temporal information on the fluorescence at a pixel level in each cell. Given the wide-spread usage of fluorescent proteins, including in studies of gene expression dynamics and cell morphology, we expect CellAging to have a wide range of applicability. Provided some modifications, CellAging may also contribute to studies of cellular aging focusing on mitochondria, given its similar shape to *E.coli* and bacterial origin and, thus, similar mechanisms associated to aging (Youle and van der Bliek, 2012). It also may be of use in studies of gene expression (Kandhavelu *et al*., 2012) or protein localization.

ACKNOWLEDGEMENT

Work funded by Fundacao para a Ciencia e a Tecnologia of Portugal (ASR, JMF), TUT President Grant (ABM), Tampere City Science Foundation (AH), and Academy of Finland (ASR).

Conflict of Interest: none declared.

REFERENCES

Carpenter,A.E. *et al*. (2006) Cellprofiler: image analysis software for identifying and quantifying cell phenotypes. *Genome Biol*., **7**, R100.

Breiman,L. *et al*. (1984) *Classification and Regression Trees*. Wadsworth, CA.

Lindner,A. *et al*. (2008) Asymmetric segregation of protein aggregates is associated with cellular aging and rejuvenation. *Proc. Natl Acad. Sci. USA*, **105**, 3076–3081.

Lloyd-Price,J. *et al*. (2012) Asymmetric disposal of individual protein aggregates in *Escherichia coli*, one aggregate at a time. *J. Bacteriol*., **194**, 1747–1752.

Kandhavelu,M. *et al*. (2012) Single-molecule dynamics of transcription of the lar promoter. *Phys. Biol*., **9**, 026004.

Mora,A. *et al*. (2011) Automated drusen detection in retinal images using analytical modelling algorithms. *Biomed. Eng. Online*, **10**, 59.

Queimadelas,C. *et al*. (2012) Protein measurements from combined dic/fluorescence images. In: *5th International Conference on MEDSIP*. Liverpool, UK.

Rokney,A. *et al*. (2009) *E. coli* transports aggregated proteins to the poles by a specific and energy-dependent process. *J. Mol. Biol*., **392**, 589–601.

Youle,R. and van der Bliek,A. (2012) Mitochondrial fission, fusion, and stress. *Science*, **337**, 1062–1065.

Young,J. *et al*. (2011) Measuring single-cell gene expression dynamics in bacteria using fluorescence time-lapse microscopy. *Nat. Protoc*, **7**, 80–88.

Yu,J. *et al*. (2006) Probing gene expression in live cells, one protein molecule at a time. *Science*, **311**, 1600–1603.