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Gene expression

MitoTrack, a user-friendly semi-automatic software for lineage tracking in living embryos

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Abstract

Motivation: During development, progenitor cells undergo multiple rounds of cellular divisions during which transcriptional programs must be faithfully propagated. Investigating the timing of transcriptional activation, which is a highly stochastic phenomenon, requires the analysis of large amounts of data. In order to perform automatic image analysis of transcriptional activation, we developed a software that segments and tracks both small and large objects, leading the user from raw data up to the results in their final form.

Results: MitoTrack is a user-friendly open-access integrated software that performs the specific dual task of reporting the precise timing of transcriptional activation while keeping lineage tree history for each nucleus of a living developing embryo. The software works automatically but provides the possibility to easily supervise, correct and validate each step.

Availability and implementation: MitoTrack is an open source Python software, embedded within a graphical user interface (download here).

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

Live imaging of developing organisms provides the unprecedented opportunity to access to nuclei/cellular genealogy. The combination of lineage information and transcriptional history permits to decipher the extent of transcriptional memory, i.e. the influence of the transcriptional status of mother nuclei on that of their daughters (Ferraro et al., 2016). Here we present MitoTrack, a user-friendly and open source software, able to automatically track nuclei and their transcriptional activity across multiple cell divisions. The tool has been primarily developed for images of living Drosophila embryos, in which labeling nascent transcription has become popular (Garcia et al., 2013; Lucas et al., 2013; Pichon et al., 2018). However, MitoTrack could also be used for genealogy and memory tracking in other organisms, such as zebrafish embryos, mouse early embryos or even in organoids.

2 Software description

The typical data processed with MitoTrack are images from early Drosophila embryos where nuclei are labeled with a histone-RFP transgene and reporter mRNA detected upon binding of the MCP-GFP to MS2 containing nascent mRNAs (sample data can be downloaded following this link) (Fernandez and Lagha, 2019). Most of MitoTrack algorithms are not novel and several more sophisticated programs are similarly able to perform lineage tracking in living organisms (Amat et al., 2014; Tinevez et al., 2017; Tran et al., 2018; Wolff et al., 2018). However, MitoTrack was developed to fulfill the specific task of extracting lineage information and the timing of transcriptional activation directly from the raw data. All the developed algorithms work in a single package, embedded into a user-friendly graphical user interface (GUI) (Fig. 1a), released as open source (a tutorial with walk-through examples is provided as a Supplementary Material). The main objective of MitoTrack is to analyze transcriptional activation in a nucleus-by-nucleus basis, across multiple nuclear divisions to quantify the extent of transcriptional memory. The general tasks are (i) to segment and track nuclei across mitosis (keeping lineage information), (ii) to segment spots (transcription sites) and (iii) to merge these pieces of information and obtain the activation timing for each mother nucleus and its daughters. The analysis is organized into four steps: 'before mitosis', 'during mitosis', 'after mitosis' and 'merging of partial analysis'.

3 Features

Input data are 4D stack (.czi or .lsm) with two channels, one for nuclei and the other for transcription sites (spots). When loaded, data are automatically maximum intensity projected.

The ‘before mitosis’ and ‘after mitosis’ steps of the analysis work similarly: nuclei are segmented and tracked while transcriptional sites

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are detected and each of them is associated to the closest -if not overlap-
ning- nucleus. Nuclei are pre-smoothed and then thresholded with the
classical Otsu thresholding algorithm (Otsu, 1979); the black and white
resulting images are labeled and segmented using a watershed segmenta-
tion algorithm (van der Walt et al., 2014) and finally tracked in con-
secutive frames following a minimum distance criterion of their
centroids positions. Spots are segmented with a blob detection algo-
rithm, namely a Laplacian of Gaussian. The resulting images are filtered
and each spot is then associated to the closest nucleus. Potential segmen-
tation errors can be corrected with a pop-up tool that allows the user to
easily modify nuclei segmentation by merging or splitting nuclei
(Fig. 1c). The ‘during mitosis’ step consists in a similar nuclei segmenta-
tion but differs in the tracking procedure, where mitotic duplication of
chromosomes must be accounted for. MitoTrack follows nuclei across
mitosis under an overlapping criterion, by projecting the mask of each
nucleus at frame t onto the following frame. In this way, two nuclei (daughters) can be associated to a single nucleus (mother), thereby creat-
ing a lineage genealogy. A pop-up tool (Fig. 1b) allows the user to
easily modify both segmentation and tracking during this particular step.

The last part of MitoTrack consists in merging the three afore-
mentioned sections in order to update nuclei labels for the entirety
of the analyzed movie encompassing mitosis. Moreover, in this step,
the software attributes sub-labels to properly distinguish the two
daughters from each other. In addition, MitoTrack allows for a spa-
tial analysis and hence records the timing of transcriptional activa-
tion within user-defined domains. All the extracted quantitative
data are organized into an .xls file.

4 Results and perspectives
The described program represents a robust analysis platform to
quantify the timing of transcriptional activation prior and after
mitosis while keeping the genealogy information. Using MitoTrack
software, we can now quantify the extent of transcriptional memory
in hundreds of nuclei from various genotypes, as recently performed
in (Dufourt et al., 2018). One of the main advantages of MitoTrack
resides in the various tools it provides for manual correction of nu-
clei tracking during mitosis (Fig. 1c). However, mainly depending
on nuclei crowding and temporal resolution, MitoTrack is able to
automatically track all nuclei during mitosis with near 100% accur-
acy (for example in mitosis from nc12 to nc13 with a temporal reso-
lution of 20 s/frame, or in mitosis from nc13 to nc14 with images
every 4 s, as the sample data accompanying the provided tutorial).

By comparing the timing of activation of neighboring nuclei, we
can now directly evaluate the extent of temporal coordination
(referred to as synchrony (Lagha et al., 2013)). Thanks to
MitoTrack spatial analysis tool, it is possible to measure the timing
of activation in distinct spatial domains and thus quantify the im-
 pact of gradients of morphogens (for example, the Dorsal activator)
on transcriptional dynamics.

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Conflict of Interest:
none declared.

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