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CAST: An automated segmentation and tracking tool for the analysis of transcriptional kinetics from single-cell time-lapse recordings

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ABSTRACT

Fluorescence and bioluminescence time-lapse imaging allows to investigate a vast range of cellular processes at single-cell or even subcellular resolution. In particular, time-lapse imaging can provide uniquely detailed information on the fine kinetics of transcription, as well as on biological oscillations such as the circadian and cell cycles. However, we face a paucity of automated methods to quantify time-lapse imaging data with single-cell precision, notably throughout multiple cell cycles. We developed CAST (Cell Automated Segmentation and Tracking platform) to automatically and robustly detect the position and size of cells or nuclei, quantify the corresponding light signals, while taking into account both cell divisions (lineage tracking) and migration events. We present here how CAST analyzes bioluminescence data from a short-lived transcriptional luciferase reporter. However, our flexible and modular implementation makes it easily adaptable to a wide variety of time-lapse recordings. We exemplify how CAST efficiently quantifies single-cell gene expression over multiple cell cycles using mouse NIH3T3 culture cells with a luminescence expression driven by the *Bmal1* promoter, a central gene of the circadian oscillator. We further illustrate how such data can be used to quantify transcriptional bursting in conditions of lengthened circadian period, revealing thereby remarkably similar bursting signature compared to the endogenous circadian condition despite marked period lengthening. In summary, we establish CAST as novel tool for the efficient segmentation, signal quantification, and tracking of time-lapse images from mammalian cell culture.

1. Introduction

Improvements in microscopy techniques has enabled rapid progress in quantitative cell biology [1–7]. Notably in mammalian cells, time-lapse imaging has provided unprecedented insights into complex problems such as transcriptional bursting [8–11], cell cycle transitions [12,13] and the circadian oscillator in individual cells [14–17]. However, a current bottleneck is that accurate quantification of time-lapse imaging data is typically slow, repetitive, and requires a significant amount of human intervention (reviewed in [18]). Therefore, versatile automated solutions to robustly quantify temporal signals reflecting noisy biological processes are needed. In particular, single-cell analysis typically requires quantifying hundreds of individual expression traces to overcome inherent stochasticity and gain statistical significance [15,16,19,20]. However, both the segmentation, i.e. the recognition of object in single frames, as well as the tracking, namely the linking of objects corresponding to the same instance over consecutive frames, are challenging computational tasks.

An intuitive and widespread method for localizing objects is to determine a threshold value in the intensity of the image to separate the signal from the background [21–23]. However, this method is particularly sensitive to fluctuations often observed in biological data, such as cell-to-cell variation, low signal images or uneven illumination. Therefore, segmentation methods based on thresholding are often used as an initial step that is refined by other algorithms such as active contours [24], watersheds [25], morphological operations [26–28], or machine learning methods [29]. In addition, segmentation algorithms that use alternative detection strategies were developed, including multi-scale wavelets [30], cross-correlations [31] or likelihood criteria [32]. Howbeit, the performances of each detection method is strongly dependent on the type of data to be segmented, in particular for images with low signal-to-noise ratio (SNR) or objects of varying sizes (reviewed in [33]).

Abbreviations: CAST, Cell Automated Segmentation and Tracking platform; OME-TIFF, Open Microscopy Environment Tagged Image File Format; MAD, Median Absolute Deviation; SNR, signal-to-noise ratio.

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After objects are localized in a sequence of time-lapse frames, tracking them across frames is also a very challenging task, which is often complicated by the motion, disappearance, fusion or splitting of the studied objects. Moreover, tracking suffers from a problem of combinatorial explosion in the number of potential assignments that renders the identification of the global optimum practically unfeasible for biological datasets. Different strategies were developed to circumvent these problems such as fitting Gaussian curves to object intensity values [34], using cross-correlation of consecutive frames [35] or calculating the probability of each set of assignment [36]. Alternatively, a number of greedy algorithms avoid the aforementioned issues by solving the frame-to-frame correspondence problem in a spatially global fashion [37–40]. However, objective comparisons between these methods concluded that these often need to be tailored to a particular problem, and typically require significant manual intervention and curation [18,41–43]. An additional challenge occurs when the signal intensity of an object drops below detectability during certain time intervals (e.g. during the cell cycle [44] or the circadian cycle [14,15]), in which case the algorithm needs to recognize and stitch the track over the resulting gaps. However, while this caveat can be alleviated by using constitutively expressed reporters in multi-channel fluorescent imaging (e.g. a nuclear or membrane marker), this is not easily possible in bioluminescence imaging, which typically permits the acquisition of only one channel.

In our ongoing work on single-cell transcription [8,9], we experienced that none of the available software tools providing implementations of such segmentation and tracking algorithms [45–56] combine the level of accuracy and automation that we are aiming for our bioluminescence data. Consequently, we set out to develop CAST (Cell Automated Segmentation and Tracking platform), a robust and automated image analysis algorithm for the segmentation, tracking and quantification of time-lapse recordings. To illustrate the potential of CAST, we analyze single-cell bioluminescence reporters engineered to study transcriptional bursting, and demonstrate how transcriptional fluctuations can be deciphered using mathematical modeling to dissect the underlying transcription process. Regardless of this particular application, CAST was conceived in a highly modular fashion, so that its implementation is versatile enough to be readily fitted to many recording configurations, including fluorescence imaging.

2. Material and methods

CAST was developed as a set of custom MATLAB functions accessed through user-friendly graphical interfaces (see Appendix A). Importantly, while we here detail its applicability on bioluminescence data, its modular implementation can be easily adapted to the analysis of a wide variety of time-lapse data. CAST consists of four main steps (described below) that are configurable, editable and, once the corresponding parameters are manually tuned to the specificity of the analyzed recording, fully automatized. Together, these provide a robust way to track the reporter signals in mammalian culture cells using a single emission channel. All code is freely available via the Github service (https://git.epfl.ch/repo/cast.git).

2.1. Preprocessing of images

For maximal compatibility between the numerous existing types of biological data, CAST starts by converting the time-lapse recording to be analyzed to a standardized format (unsigned 16-bits OME-TIFF stacks, [57]). Three filtering steps can then be applied to the resulting images. First, a background correction can compensate for non-uniform illumination of the images. The background is obtained, for each image of the recording independently, by morphologically opening the raw image, smoothing it using a Gaussian kernel, and least-squares fitting of a 2D quadratic surface on the resulting intensities. Of note, the fitted background, that is then subtracted to the raw image, is centered (i.e. has a mean of zero) to remove trends from the image without altering the range of values. Second, CAST can remove saturated pixels from cosmic rays (useful for bioluminescence when long exposure times are used) with a local histogram approach [58], using robust statistics for the estimation of the location and scale (i.e. using the median instead of the mean and the spell out, MAD, instead of the standard deviation). Thirdly, CAST can remove defective pixels by detecting them as a signal varying more from the mean of the whole image than a threshold defined times the standard deviation. Finally, the entire stack of images can be normalized (i.e. scaled) to compensate for variation between recordings. The result of all these operations is then stored in a new image stack that is used for all the subsequent steps of the analysis.

2.2. Image segmentation

The steps described below are optimized for detection of spherical objects as typically encountered in bioluminescence imaging (e.g. cells, nuclei or other organelles depending on the type of reporters and image resolution), however, these steps can straightforwardly be adapted to segment different types of objects (e.g. non-spherical). As the first step towards segmentation, a background subtraction (as described in 2.1) can be applied, here affecting only this step of the analysis. Second, default de-noising is performed by applying a Gaussian filter with a radius of 0.6 px [59] and by subtracting the estimated mean of the uniform Gaussian white noise, replacing the homogeneity analyzer proposed in [60,61], which is utilized to identify the empty portions of the image, by the Absolute Difference Mask (ADM) edge detector [62]. Candidate object locations are then detected using the "à trous" wavelet transform [30], followed by a filtering step in which only single-pixel local maxima detections are kept. The bioluminescence signal corresponding to each of the detected position is then estimated by iterated least-square fitting of a 2D symmetric Gaussian function (based on [63]). Spurious detections are then filtered out using size and intensity thresholds (all thresholds come with default values but can be changed by the user). Finally, overlapping cells are fused together. The position of the new cell substituting the fused objects is estimated using a weighted average of their respective positions, each weight being proportional to the integral of the corresponding signal intensity. The parameters of the signal are then similarly computed by a weighted average of the fused signals, using a Gaussian weighting kernel of their respective distance to the averaged position.

2.3. Cell tracking

Tracking of cells is performed in CAST using a custom, more memory efficient, implementation of an algorithm that solves both the frame-to-frame linking of objects and the problem of assigning trajectories globally using the Hungarian algorithm [40]. Our implementation uses sparse matrices for the previously proposed cost matrices to decrease the memory load of the algorithm, a major bottleneck in the standard implementation, hence permitting the efficient handling of a very large number of trajectories (i.e. tested on more than 5000 trajectories). Thus, CAST can efficiently handle the gap closing (i.e. linking similar objects that were not segmented in some frames), merging and splitting steps