Supplementary Materials and Methods

Cells, sample preparation and microscopy

The GFP-MYC mouse line has been described previously (1). For time-lapse video microscopy 200,000 ESCs were seeded in 3.5-mm 0.1% gelatine-coated plastic plates and cultured in 2xLIF SR-ES medium. 24 hours later cells were transferred to the environmental chamber of a LSM 780 upright microscope Zeiss (Jena, Germany) stabilised to 37ºC and 5% CO2. To avoid liquid evaporation, embryo-grade mineral oil was added on top of the medium after objective immersion. Samples were imaged with a W Plan-Apochromat 40x/1.0 DIC M27 objective at 0.415x0.415µm resolution (512x512 pixels) every 7 minutes for 24 hours. Z-stacks with sections spaced at 2 µm were acquired at each timepoint. In addition, a multi-position map was generated; therefore every ESC colony was manually assigned an x-y-z location. A trade-off between spatial resolution, temporal resolution and total acquisition timing was carefully chosen in order to minimize photobleaching and phototoxicity while being able to track cell division, apoptosis and cell-cell interactions. A total of 6 time lapse experiments (24 h. each) were acquired and analysed except for the evaluation of execution times and performances. For the evaluation of ESC-T versus TrackMate, a subset of a video with 1.4 hours, 12 frames and 14 cells was cropped. For the evaluation of execution times versus the size of videos, 5 subsets of videos with 14 cells in average were generated with 12, 25, 51, 103 and 206 frames respectively. An additional video with 412 frames was synthetically created from an original one by applying temporal interpolation.

Implementation resources

Image processing including segmentation, tracking and analysis methods were developed using Definiens Developer software version XD2.4 - Definiens AG (Munich, Germany) . The data analysis and lineage tracing pipeline was developed in MATLAB (R2015a, toolboxes Bioinformatics, Image Processing, Optimization, Statistics and Machine Learning))- Mathworks (Natick, MA, USA). MATLAB Tree Data Structure Toolbox is used in ESC-T data analysis and lineage tracing pipeline. The MATLAB Tree Data Structure Toolbox was published by J.Y. Tivenez (https://es.mathworks.com/matlabcentral/fileexchange/35623-tree-data-structure-as-a-matlab-class, http://tinevez.github.io/matlab-tree/)

Execution parameters

TrackMate: LoG Detector with estimated blob diameter 100, threshold 1, without median filter was used on the nuclear Myc intensity channel. LAP tracker with track segment splitting of 15 um only, no gap closing, was used. Spherical detection was used as the segmentation result. TrackMate was run into a Intel(R) Xeon(R) CPU E5-1620 v2 3.70GHz 16GB RAM with Windows 7 Professional 64 bit.

ESC-T: Definiens ruleset was run into a Xeon X5555 2 CPU - 4 cores 2.67GHz 12GB RAM with Windows Server 2012 R2.

Segmentation and tracking accuracy evaluation

The evaluation of accuracy of segmentation (SEG) and tracking (TRA) was carried out as proposed at the cell tracking challenge (2). The software provided at the challenge website was directly used to obtain a segmentation accuracy measurement “SEG” which is in the 0,1 interval, where 1 means perfect match and 0 means no match. Tracking accuracy measurement “TRA” is normalized in the 0,1 interval, where higher values of TRA reveal better tracking performance. Further detail on the metrics can be found in the Challenge website :

(http://www.codesolorzano.com/celltrackingchallenge/Cell_Tracking_Challenge/Metrics.html).

Statistical Analysis

Linear correlation coefficient and p-value in Fig. 4b were conducted using Pearson's correlation and a Student's t distribution. Mann-Whitney U test was performed in Fig. 4 c, d and g. In Fig. 4c two groups were considered for the statistical analysis, one formed by cells with distance to mitosis less than 1 hour, and the other containing cells with distance to mitosis equal or more than 1 hour. In Fig. 4d, dividing and non-dividing cells were compared for all the distances to the born of the cells in terms of MeanMyc_Neighbours_Compensated, (Table S1). In all tests, statistical significance p-value for H0 rejection was 0.001.

Normalization of mean Myc values compensating for intensity loss along time

During time-lapse analysis, photobleaching of GFP-MYC fluorescence produced a progressive signal reduction throughout the first third of the tracking period. After the initial photobleaching, the GFP-MYC signal reached the horizontal asymptote and was stable during the remainder of the tracking period (Fig. S1a). The compensation of fluorescence intensity loss along time was necessary to avoid interference from photobleaching during the analysis of cellular features related to GFP-MYC fluorescence intensity during the lifetime of cells. We used a normalization technique based on the method described in (3), which uses a post processing method to compensate images after captured motivated by histogram matching of the profiles of foreground probability distributions along timelapse. Other works have used this approach (4) since it presents multiple advantages: by matching foreground and not entire image, the method avoids the problems arising due to change in structure of the image. Furthermore, the approach does not use the model of an intensity decay function of depth or time (IDF) so that it can be applied to microscopy imaging system suffering intensity loss due to deteriorating factors that cannot be modelled accurately with IDF as occurring in our particular application, where the combination of intensity loss due to photobleaching, depth and time can give rise to a complicated IDF. We adopted a compensation using histogram matching applied to nuclei Myc mean values once nuclei segmentation has been done instead of pixel-based compensation as reported on the method described in (3). Formulation proposed remains as follows:

$$x_i^{t\prime} = \left\{ \frac{(x_i^t - mean^t)}{std^t} std^r + mean^r \right\} \qquad \text{[Eq. 1]}$$

Where x_i^t is the mean intensity for cell *i* in timepoint *t*, $x_i^{t\prime}$ is the normalized mean intensity for cell *i* in timepoint *t*; $mean^t$ is the mean of intensities in *t*; std^t is the standard deviation of intensities in *t*; $mean^r$ and std^r are the mean and standard deviation of intensities in the reference time point (last timepoint, when photo-bleaching reaches stabilization) (Fig. S1).

Data mining; Lineage-dependent measurements

Once the tracking is completed for an entire image dataset, aggregated measurements that use the whole cell track information are computed for complete cell lifetimes, from the initial to end timepoints. These include mean, median, standard deviation, maxima and minima of GFP-Myc intensity, object roundness, among others (Table S1). Additional lineage-associated features can be extracted to provide genealogical information for lineage tracing and lineage-dependent measurements. Lineage events are computed at each specific timepoint and include Boolean scoring of mitosis and apoptosis, born and dividing. Numerical identifier of progenitor cell (predecessor ID) as well as number of cell divisions are computed for each cell in all timepoints. Time to birth, to division and to death are computed for every cell at each time point and allow *in silico* synchronization of the cell cycle. Lineage event scores like apoptotic or dividing allow aggregated measurements per cell/family such as apoptotic or mitotic cells/families of GFP-Myc intensity, object roundness, etc.

Motility related features include 3D information about the movement of cells using the center of mass of segmented objects at each timepoint. Filtered scoring of the following parameters is also computed by local smoothing of the paths of each cell/family: Velocity, angle of displacement, Euclidean distances and accumulated walking distances between chosen timepoints (Table S1).

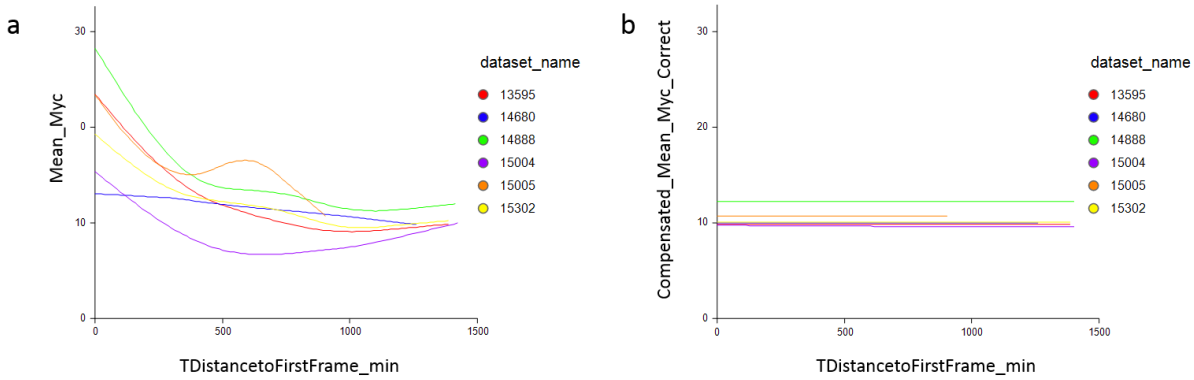

Fig. S1. Normalization of mean Myc values with compensation for intensity loss over time. Mean myc values for every nuclei in a dataset with respect to the time to first frame before (a) and after (b) compensation for bleaching. Locally weighted scatterplot smoothing is applied.

Supplementary Materials and Methods References

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3. **Gopinath, S., Q. Wen, N. Thakoor, K. Luby-Phelps, and J.X. Gao**. 2008. A statistical approach for intensity loss compensation of confocal microscopy images. J. Microsc. *230*:143–159.

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