Robust image processing techniques for tracking and cell shape outlining: Application to mammalian circadian behaviour of cells

Charna Dibner[1], Daniel Sage[2], Ueli Schibler[1], Michael Unser[2]

[1]*Department of Molecular Biology, University of Geneva, Geneva, Switzerland*
[2]*Biomedical Imaging Group, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*

The mammalian circadian timing system has a hierarchical structure in that a master pacemaker residing in the suprachiasmatic nucleus (SCN) synchronizes slave oscillators existing in most body cells. While oscillators in SCN neurons are known to function in a self-sustained fashion, rhythms of peripheral oscillators have been thought to damp rapidly when disconnected from the control exerted by the SCN. Recent advances in time-lapse fluorescence imaging have provided an important tool to study the mechanisms underlying circadian clock work at the individual cell level under different conditions. Transgenic NIH 3T3 cell line stably expressing a short-lived and nuclear yellow fluorescent protein (VNP) from circadian regulatory elements of the *Rev-erbα* locus was recently established in Ueli Schibler's lab and successfully used to unravel interactions between the circadian clock and the cell division clock. However, existing computational image processing methods are rather limited in analyzing and tracking such time-lapse datasets, and manual analysis is unreasonably time-consuming and subject to errors in observer judgement.

This led us to the development of a new user-friendly image-analysis software for accurate tracking of individual in a living cell population imaged over a few days. In these fluorescence sequence images, the cells are characterized by a small frame-to-frame displacement, a slow evolution of their shapes and a high variation of fluorescence intensity. In some cases, the intensities of the cells are so low that they are almost indistinguishable from the background. Our software allows the user to specify the cells of interest. In addition, the user is allowed to mark the cell position in a few images in the sequence. Typically, the user is required to indicate the cell position in one frame out of every 50 frames. The software proceeds in three steps: 1) **Pre-processing:** We apply a non-linear diffusion filter and rescale the images to homogenize the intensities of the cells. Pre-processing is done only once for a given sequence of images. 2) **Tracking:** We compute the temporal trajectory of the cell from a down-sampled version of the images. Down-sampling the image reduces the computation time. We use a dynamic programming algorithm (DPA) for finding the optimal path that links the user-specified time-positions under the additional constraint of a small displacement from one frame to another. The main advantage of DPA is that it helps us track the cell even when the intensity is low and the cell is out-of-focus. 3) **Cell shape outlining:** In this step, we outline the cell accurately for each frame with a closed curve, using the gradient-based edge image. At this point, the cell is completely segmented over the entire sequence of images. The software then computes a set of parameters that describe the cell: the cell size, mean intensity, displacement and direction of the path. Thus, we achieve a quantitative description of the cell behaviour. The results are reported in the form of tables and graphs for a circadian cycle. The software was developed as a plug-in for *ImageJ,* a Java-based image processing software. The software is efficient and fast enough to allow the user to edit the trajectory and restart the process of tracking the cell and outlining its shape.

The experimental results show that our new technique is reliable, reproducible and efficient for individual cell tracking. The software is a valuable tool in bioluminescence time-lapse microscopy applications and circadian analysis.