AUTOMATED TRACKING OF SINGLE FLUORESCENCE PARTICLE

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INTRODUCTION: We present a novel and robust computational procedure for tracking fluorescent particles in images of time-lapse microscopy. The algorithm is optimized for finding the trajectory of single particles in very noisy dynamic image sequences. We have applied the software to trace the movement of chromosomal telomeres within the nucleus of a yeast cell [1]. The new algorithm reduces the analysis time of a 300 images sequence from 10 minutes, when it is done manually, to just a few seconds. It also offers the benefit of reproducibility.

METHODS: The goal is to obtain a complete, reliable description of the trajectory of a particle from a sequence of noisy images (Fig 1).

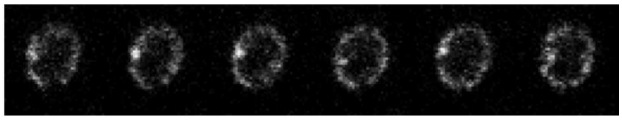

Fig. 1. Six images out of a sequence of 300. The brightest spot (particle to track) moves inside the cell nucleus. Notice that the nucleus membrane is also marked by the fluorescence.

We have developed an automated tracking procedure [2], which proceeds in three steps.
1) *Sequence alignment*. We are interested in characterizing the movement of the fluorescent spot in relation to the nucleus membrane, which is fluorescently labeled and visible in the image. In practice, the nucleus does not necessarily remain stationary during the acquisition. Therefore, we have to compensate for this effect by fitting an ellipse to the thresholded data, using a least squares method. Afterwards, we realign the images. 2) *Spot enhancing filter*. To improve the robustness of the tracking algorithm, a pre-filter is applied to enhance the particles while suppressing the background and reducing the effect of noise. We have shown that the optimal filter for the detection of a particle (assuming a Gaussian shape) in presence of additive noise with a spectral power density function that decays like $O(\omega^{-2})$, is the Laplacian of a Gaussian (LoG), also known as the "Mexican Hat" filter. 3) *Tracking*. The key feature that makes the method robust and foolproof is our formulation of the detection task as a global optimization problem; i.e., over the whole sequence of images instead of a frame-to-frame approach. The optimal solution is computed efficiently by a dynamic programming procedure. The global optimum satisfies a displacement constraint and minimizes a cost function that favors positions with high intensity and penalizes non-smooth trajectories. The whole process is implemented as a versatile Java plug-in for the popular public-domain ImageJ software [3]. The algorithm is fast enough for an application for which the images are small (0.6s. for 200 images of 50x50 pixels).

RESULTS: The results obtained on real data (Fig. 2) are as good—if not better—as manual tracings. The plug-in turns out to be a considerable help to the biologist since it allows for fast, reproducible and reliable analysis of a large number of experiments.

DISCUSSION & CONCLUSIONS: We have proposed an automated tracking procedure to trace single fluorescence particles in a sequence of noisy images. The procedure is an attractive alternative compared to manual tracing, which is much more laborious and less reproducible. We applied the algorithm successfully to the analysis of the movement of chromosomal loci within the nucleus of a yeast cell. The results obtained are highly satisfactory, suggesting that the dynamic programming approach has good potential for similar biological imaging problem. Our software is generic and is applicable to similar biological imaging applications

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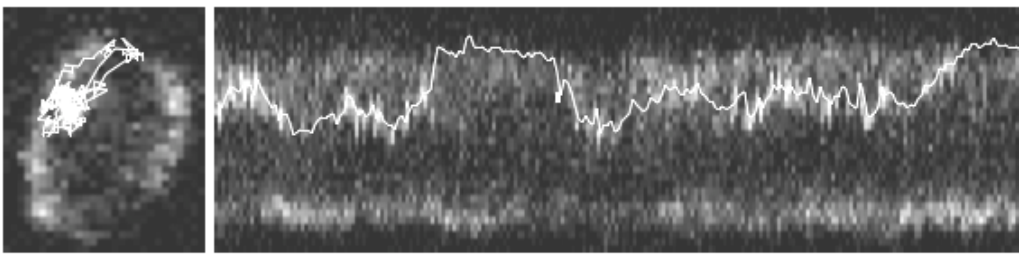

*Fig. 2. Tracking result of a tracking over a sequence of 228 images (28*34 pixels). The left image is the xy section at t=1. The right image is a y(t) orthogonal section. The overlaid line is the projected trajectory of the spot. Here, the limited displacement constraint 3 pixels.*