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Review

Tracking in cell and developmental biology

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ABSTRACT

The past decade has seen an unprecedented data explosion in biology. It has become evident that in order to take full advantage of the potential wealth of information hidden in the data produced by even a single experiment, visual inspection and manual analysis are no longer adequate. To ensure efficiency, consistency, and completeness in data processing and analysis, computational tools are essential. Of particular importance to many modern live-cell imaging experiments is the ability to automatically track and analyze the motion of objects in time-lapse microscopy images. This article surveys the recent literature in this area. Covering all scales of microscopic observation, from cells, down to molecules, and up to entire organisms, it discusses the latest trends and successes in the development and application of computerized tracking methods in cell and developmental biology.

Contents

1. Introduction

It has been increasingly recognized in recent times that life is a miraculous symphony [1]. From fast metabolic pathways to the cell cycle, to the beating of the heart, all the way to annually repeating seasonal behaviors, life is composed of a multitude of interconnected oscillations, together constituting a gigantic orchestra spanning at least 10 orders of time magnitude. Recent investigations seem to suggest that careful coordination of these rhythms and their interactions is an important precondition for the maintenance of normal development and health. Conversely, a disturbance at any level of this intricate time network can be expected to result in disease. Although it is not our purpose here to discuss the interesting findings of chronobiological investigations, they do emphasize the importance of studying life's processes

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in both space and time [2], that is, to analyze their structure and function.

The ability to visualize cells and subcellular dynamic processes in space and time has been made possible by revolutionary developments in imaging technology in the past two decades. Advances in molecular biology, organic chemistry, and materials science have resulted in an impressive toolbox of fluorescent proteins (GFP and variants) and nanocrystals (quantum dots), and have enabled the study of protein expression, localization, conformation, diffusion, turnover, trafficking, and interaction [3,4]. On the hardware side, advances in optical systems design have taken light microscopy from widefield to (multiphoton) confocal and spinning disk microscopy [5,6], and more recent efforts to break the diffraction barrier have further extended the palette [7,8]. Together, these developments have redefined biological research by enabling the switch from fixed to living cells and from qualitative to quantitative imaging [2,9].

As was to be expected, the new possibilities offered by these developments to image (sub)cellular processes in space, time, and at multiple wavelengths, have resulted in a true data explosion. It has now become evident that in order to ensure efficiency, consistency, and completeness in handling and examining the wealth of image data acquired in even a single experiment, computational image management, processing, and analysis methods are indispensable [10–18]. Thus, it seems that the bottleneck in putting modern imaging technologies to high-throughput use, has shifted from the "wetware" and the hardware to the development of adequate software tools and data models. While the need for such tools has been recognized for a long time in the medical imaging communities, and advanced image processing, computer vision, and pattern recognition methods have been developed in the past 30 years to enable computer-assisted diagnosis in various clinical applications [19–21], it is only since relatively recently that similar methods are being explored to facilitate automated image analysis in biological imaging [22,23].

This article briefly surveys the latest trends and successes in the endeavor to take full advantage of the vast amounts of image data acquired in biological imaging experiments. The emphasis is on tracking and motion analysis of objects in time-lapse microscopy images. Updating previous surveys, aimed at engineers [16,24,25] or biologists [17,26,27] from different perspectives, we cover tracking at all scales of microscopic observation, from molecules, to cells, to organisms. In view of the rapid developments in the field, and because of space limitations in the present article, we consider only (a subset of) works published since the year 2000. First, we give an overview of recent cell segmentation and tracking algorithms, which in many experiments constitute the basis for further analyses. In the subsequent sections, we shift focus in two possible directions: from cells down to molecules (capturing the trajectories of intracellular particles), and from cells up to organisms (following embryogenesis and adult locomotory behavior). The article hopefully serves as a useful source of pointers to the relevant (mostly methodological) literature on tracking for a wide variety of applications in cell and developmental biology.

2. Cell tracking

Being the fundamental units of life, cells are the key actors in many biological processes. Cell proliferation, differentiation, and migration are essential for the conception, development, and maintenance of any living organism. These processes also play a crucial role in the onset and progression of many diseases. Understanding physiological processes in health and disease and developing adequate drugs requires the imaging and analysis of the (morpho)dynamic behavior of single cells or cells in tissues under normal and perturbed conditions [28]. This typically involves the tracking and quantification of large numbers of cells in time-lapse fluorescence, phase-contrast, or intravital microscopy data sets consisting of hundreds to many thousands of image frames, making manual analysis no option, especially in 3D.

The automation of these tasks faces several challenges, including the generally poor image quality (low contrast and high noise levels), the varying density of cell populations due to division and cells entering or leaving the field of view, and the possibility of cells touching each other without showing sufficient image contrast. Many computerized methods for cell tracking have already been proposed, and some of these have found their way to commercial and open-source software tools (summarized in [29]), but the consensus arising from the literature seems to be that any specific tracking task requires dedicated (combinations of) algorithms to obtain optimal results. Nevertheless, several trends can be observed in the development of new cell tracking methods, suggesting the superiority of particular algorithms.

2.1. Segmenting individual cells

Cell tracking methods generally consist of two main image processing steps: (1) cell segmentation (the spatial aspect of tracking), and (2) cell association (the temporal aspect). Segmentation is the process of dividing an image into (biologically) meaningful parts (segments), resulting in a new image containing for each pixel a label indicating to which segment it belongs (such as "foreground" versus "background"). One approach to segmentation is to compare the value of each image pixel to a preset threshold value and to label pixels with values above (below) the threshold as foreground (background) [22]. Due to its simplicity, thresholding is one of the most commonly used segmentation methods, but it is also one of the most error-prone [30]. It will be successful only if cells are well separated and their intensities differ sufficiently and consistently from the background—a condition hardly ever met in live-cell imaging due to severe noise, autofluorescence and photobleaching (in the case of fluorescence microscopy), or strongly varying intensities and halos (in the case of phase- or differential interference contrast microscopy).

More sophisticated methods for cell segmentation include fitting predetermined cell intensity profiles (templates) to the image data. This template matching approach works well for images showing consistent cell shape [31], but fails in the case of significant variations in cell morphology (between cells per image, or per cell over time, or both). A more popular approach is to use the so-called watershed transform. In this case, an image is considered a topographic relief, and "flooding" this relief from the local intensity minima completely subdivides the image into regions and delimiting contours, by analogy termed "catchment basins" and "watersheds", respectively. The most important drawbacks of this method are its sensitivity to noise and its tendency to yield too fragmented results (oversegmentation). Nevertheless, by combination with carefully designed pre- and postprocessing strategies, such as marking and model-based segment merging, the method has been successfully applied to cell segmentation in microscopy [32–35].

Recent years have shown an increasing interest in the use of deformable models for cell segmentation [36–46]. These are defined either explicitly as parametric contours (mostly for 2D applications) or implicitly as the zero-level of a so-called level-set function (a mathematical concept that can be applied to image data of any dimensionality). The latter approach is often preferred, as it can naturally capture topological changes, such as cell division. Starting from a coarse, initial segmentation, deformable models are iteratively evolved in the image domain to minimize a predefined energy functional (Fig. 1). The modeling aspect lies primarily in the definition of this energy functional. Typically it consists of

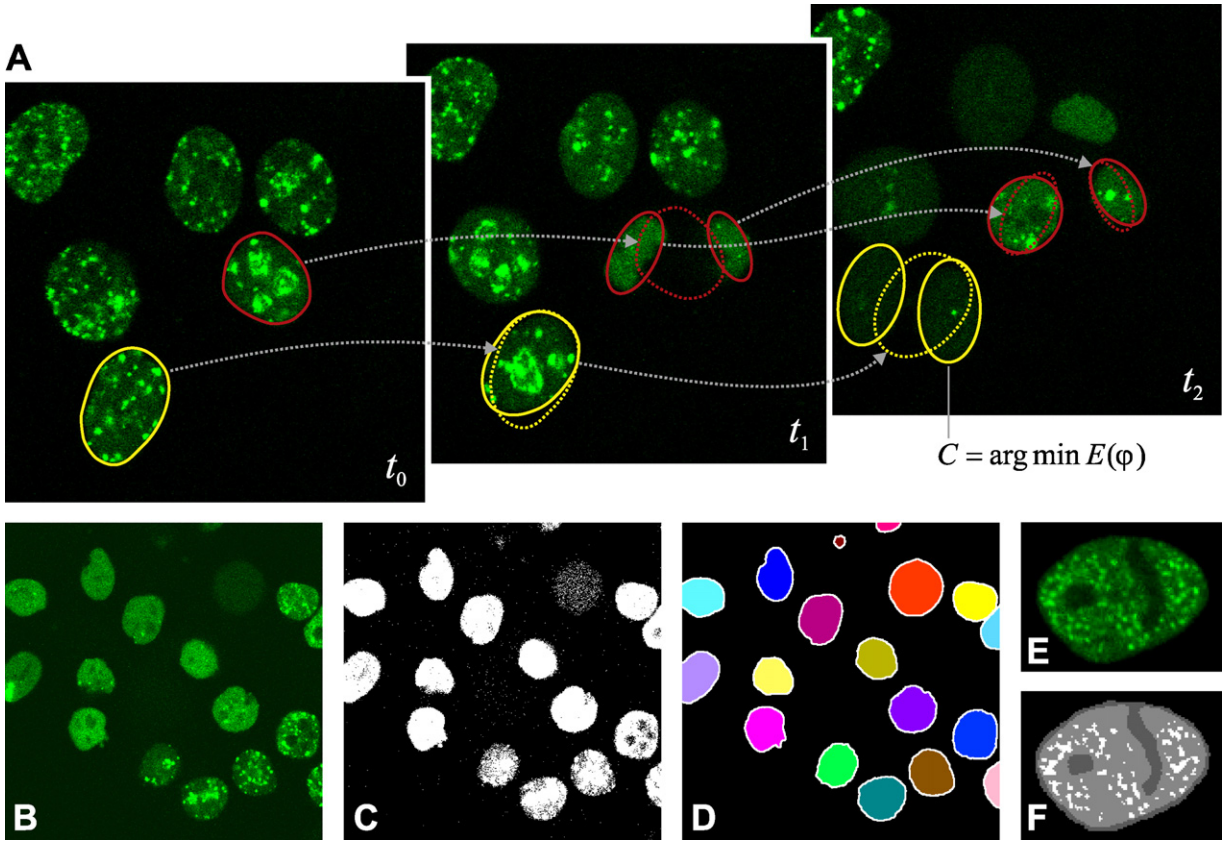

Fig. 1. Cell tracking. (A) Three frames from a time-lapse fluorescence microscopy image, illustrating the concept of model-evolution based cell segmentation and association. Cell contours or surfaces can be defined parametrically or as the zero-level of a higher-dimensional function. In each image frame, the final contour of a cell (solid line) is obtained by minimization of an energy functional, typically consisting of image-based and shape-based terms. The initial cell contour (dotted line) for each frame is usually taken to be the final contour from the previous frame. Cell divisions can be detected by monitoring the shape of the contour function during energy minimization. (B) Depending on the type of labeling, the appearance of cells (or in this case their nuclei) may vary greatly, within one frame as well as over time. (C) Thresholding usually results in a very noisy (at low thresholds) or fragmented (at high thresholds) segmentation. (D) Model-based segmentation (in this case using level sets) can yield much more sensible results. Once all cells are tracked, they can be easily extracted individually (E) and geometrically transformed to a reference coordinate-frame for subsequent intracellular analysis (F).

image-related terms (based on image features such as intensity, gradients, and texture) and image-independent terms (based on shape properties such as boundary length or surface area, curvature, and similarity to reference shapes). This mixture of terms enables the incorporation of both image information and prior knowledge about the biological application [25]. In contrast with the watershed transform, model-evolution approaches have the tendency to yield undersegmentation of the images (the contours or surfaces of neighboring cells may easily merge in the process), and usually require postprocessing steps to refine the results.

2.2. Connecting cells over time

After segmentation, the second step in achieving cell tracking is cell association. This refers to the process of identifying and linking segmented cells from frame to frame in the image sequence to obtain cell trajectories. The simplest approach to accomplish this is to associate each cell in any frame to the spatially nearest cell in the next frame (for example according to centroid position) within a predefined range. However, when dealing with many cells or rapid cell movements, this may easily lead to mismatches. In order to allow for better discrimination of potential matches, the definition of "nearest" may be extended to include similarity in (average) intensity, area or volume, perimeter or surface area, orientation of major and minor axes, boundary curvature, estimated displacement, and other features. Increasing the number of features used for comparison reduces the risk of ambiguity. A similar argument applies when using so-called mean-shift processes to iteratively compute cell positions [47,48].

Several of the discussed methods for cell segmentation can naturally be extended to also perform cell association. The concept of template matching, for example, can serve as a basis for image registration between time points. Registration refers to the process of (global or local) alignment of images, using intensity- or geometry-based features. This can be done at the cell level [49], at the level of feature points [50], or down to the pixel level, reminiscent of optic-flow schemes [29,51]. In the case of deformable models, cell association can be performed "on the fly", by using the segmentation results in any frame as initialization for the segmentation process in the next frame (Fig. 1). Essentially, this is again a nearest-neighbor linking approach, which works well if the population density is not too high and the rate at which images are acquired is such that cells do not move more than at most their diameter between consecutive frames. If these conditions are not met, or they need to be compromised for practical reasons, more sophistication is required, such as the use of gradient-vector flows [36,52], estimated cell dynamics [39,42], and special procedures to handle cell contacts, divisions, appearances, and disappearances [41,53]. Another trend in the attempt to achieve more robustness is the use of probabilistic schemes [31,42,44,54].

2.3. Cell tracking in practice

Once the cells are properly segmented in all frames of a time-lapse image sequence, and the correct associations have

been established between successive frames, it is relatively easy to compute (morpho)dynamic features that may reveal biologically relevant phenomena. For example, variabilities in cell shape (within populations or per cell over time) can be very effectively studied using statistical tools such as principal component analysis (PCA) of the cell outlines [55]. Power spectrum analysis of size changes over time have revealed the presence of regularities (periodicities hinting at underlying biochemical clocks) in the membrane deformation of crawling amoebae that were not obvious from visual inspection [25]. Automated measurement of cell motility and proliferation have enabled the study of factors influencing osteoblast differentiation and growth, involved in the processes of bone formation and maintenance, as well as the dysregulation of these processes leading to osteoporosis [56]. As a final example, computerized analysis of cell velocities, directional persistence, turning frequencies, and invasion profiles, have demonstrated significant dependence of tumor cell infiltration and migration on cell type and the microenvironment, suggesting that metastasis is not a completely random phenomenon [57]. These examples clearly illustrate that computerized cell tracking not only provides objective numbers rather than subjective visual impressions, but also offers a level of sensitivity and statistical power unattainable by human observers.

3. From cells to molecules

The capacity of cells to perform their fundamental roles in living organisms is the product of a complex machinery of intracellular and intranuclear processes, involving thousands of proteins and other constructs. Spurred by the technological advances mentioned in the introduction, the quest to improve medicine is therefore increasingly focussing on acquiring a deeper understanding of these processes. In turn, this has boosted the demand for powerful image processing tools able to automatically compute the location, distribution, and dynamics of large numbers of macromolecules in (usually fluorescence) microscopy image sequences. Going beyond ensemble averages of motion parameters, measured by fluorescence recovery after (or loss in) photobleaching (FRAP or FLIP) experiments [5,58], these offer the possibility to study dynamic processes at the highest possible level of detail (individual particles), and are often collectively (and somewhat misleadingly) referred to as "single-particle tracking" tools [24,59].

3.1. Detecting single particles

Similar to cell tracking methods, particle tracking methods too generally consist of two main processing steps: (1) particle detection (within each time frame of a sequence), and (2) particle linking (between successive frames). The former refers to the process of determining the presence and the location of particles in the images. Since individual particles (or, equivalently, the fluorescent tags used to visualize them) are typically one or two orders of magnitude smaller than the optical resolution of the microscope, they have the appearance of diffraction-limited spots ("foci", Fig. 2). In spite of this, recent studies [60] have demonstrated that for an individual particle, a localization accuracy of around 10 nm is achievable in practice, and that two or more particles can be resolved with a reasonable level of accuracy for distances of about 50 nm and larger. The limiting factor here is the photon count, or effectively the signal-to-noise ratio (SNR), which should be as high as possible to maximize estimation accuracy and precision, but in live-cell imaging experiments is usually rather low to minimize photobleaching and photodamage.

The simplest approach to particle detection is to first identify (potential) particle loci by applying intensity thresholding and then to estimate their positions by computing the centroid (center of intensity mass) for each locus [61]. For similar reasons as mentioned in the previous section, this is a frequently used but noise-sensitive approach, with limited applicability to low-SNR live-cell imaging experiments [30]. A more sensible approach is to search for loci with intensity profiles that fit the theoretical profile of a particle. For subresolution particles, the latter is equal to the point-spread function (PSF) of the microscope, which can be well approximated by the Gaussian function [62]. Experiments have shown superior performance of Gaussian fitting (with a limit of SNR ≈ 4) [63] and successful application to the detection of single [64] as well as clustered particles (using Gaussian mixture models) [65]. More sophisticated methods involve (in order of increasing performance [66]) wavelet multiscale products [67], Laplacian-of-Gaussian filtering [68], specialized algorithms from mathematical morphology [69], and machine-learning [70].

3.2. Linking particles over time

As for the linking of particles between consecutive frames, one of the simplest and most commonly used approaches is to apply the previously mentioned "nearest-neighbor" criterion. Similar to cell association, however, this quickly leads to ambiguities in the case of high particle densities and velocities [30,71]. Since individual particles usually appear as nearly identical (PSF-shaped) spots, rather than more extended regions with possibly varying shape and texture as in the case of cells, the use of additional cues to disambiguate potential matches is mostly limited to comparing particle intensities and motion consistencies over time. The linking problem is further complicated by the fact that the total number of particles is usually not constant, but may vary over time due to splitting and merging events, particles entering and leaving the field of view, disassembly and *de novo* assembly, or intermitting fluorescence (as in the case of quantum dots). In this situation, the only solution is to abandon the idea of matching on a per-particle and per-frame basis, and to develop more global linking strategies. One way to do this is to consider the tracking problem as a spatiotemporal segmentation problem (Fig. 2) and to search for optimal paths (typically tubular structures) through the entire image data [68,72,73]. An alternative (but related) approach is to construct a so-called weighted graph (or a cost matrix) from the detected particles and possible correspondences (between two or more frames) with their likelihoods, and to find the optimal subgraph (corresponding to the set of trajectories) by minimizing a global cost function defined on the graph [74–76].

Another very important trend emerging from the recent literature on particle tracking, anticipated a few years ago [24], is the paradigm shift from deterministic to probabilistic approaches. In deterministic tracking schemes, such as many of the ones discussed above, hard decisions are made at every step about the presence or absence of particles and their correspondences over time. In live-cell imaging experiments, however, there can be a great deal of uncertainty about these decisions. Regardless of the criteria used to detect or link particles, it makes sense to assign probabilities to potential particle objects and potential particle correspondences to reflect this uncertainty, and to retain all this information during tracking until the final stage, where hard decisions may (or may not) be required to allow further analyses.

Generally, probabilistic tracking methods [67,69,77–79] implement the concept of Bayesian estimation (Fig. 2), which deals with the problem of inferring the true state of a dynamic system from noisy measurements (observations) of that system. In this scheme, the "state" is described by a mathematical vector, which includes all information to be estimated (such as object position, shape, intensity, and velocity). The estimation is a recursive two-step procedure of (1) state prediction (using dynamics models based on a priori

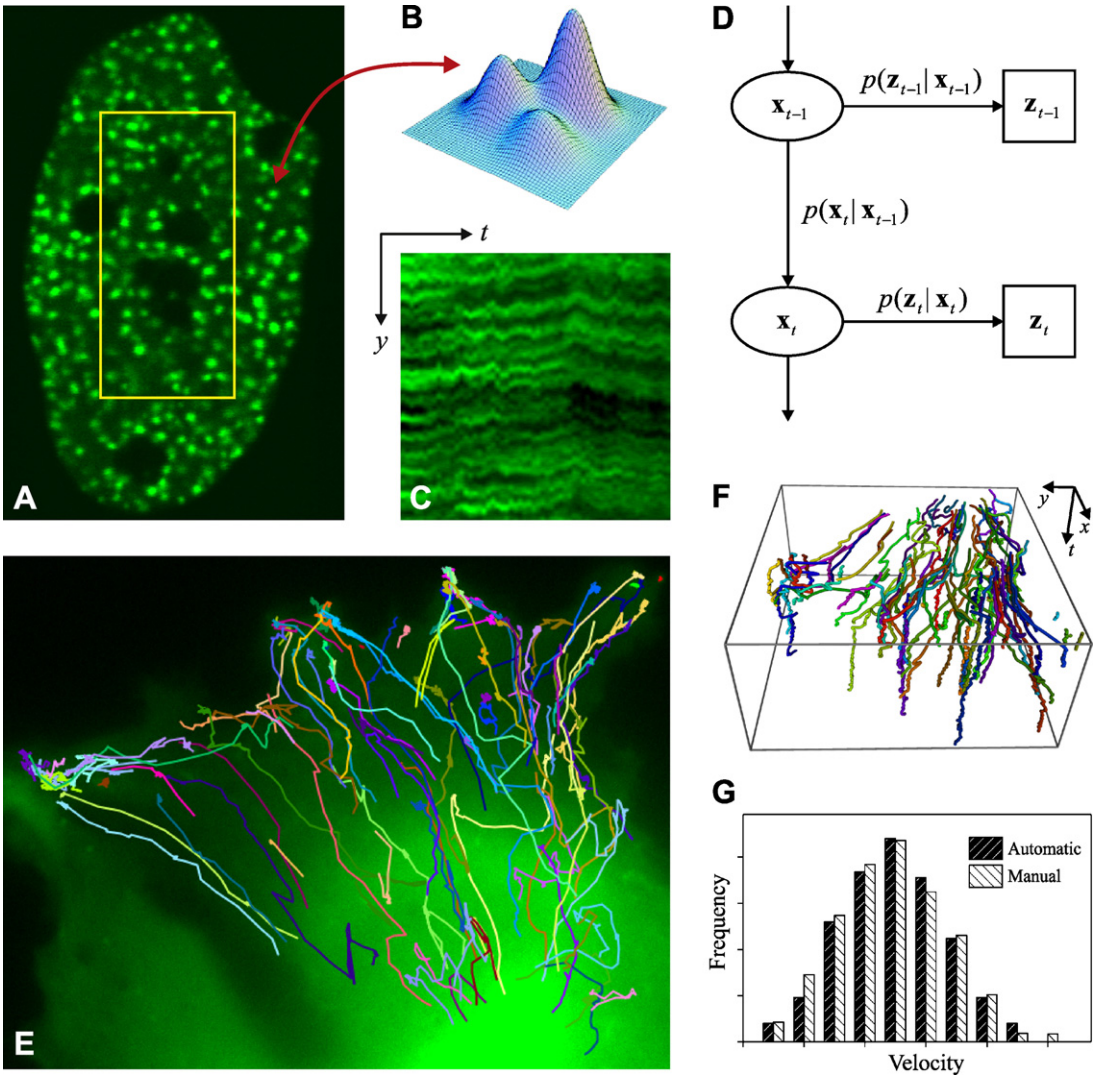

Fig. 2. Particle tracking. (A) Single frame from a time-lapse fluorescence microscopy image, showing hundreds of proliferating cell nuclear antigen (PCNA) foci in the process of DNA replication and double-strand break repair. (B) Individual foci often appear as diffraction-limited spots, which are well approximated by Gaussian (mixture) models, to be fitted to the data for detection. (C) Spatiotemporal projection image of the yellow rectangle in (A), illustrating that moving particles give rise to tubular structures in space-time, possibly extractable by spatiotemporal segmentation methods. (D) Bayesian network of the hidden Markov model assumed by probabilistic tracking methods. The posterior probability $p(\mathbf{x}_t|\mathbf{z}_t)$ of the true state $\mathbf{x}_t$ of the system (containing all relevant object features) given all measurements $\mathbf{z}_t$, is obtained by prediction based on the posterior of the previous time step and the dynamics model $p(\mathbf{x}_t|\mathbf{x}_{t-1})$, followed by update of the prediction based on the observation model $p(\mathbf{z}_t|\mathbf{x}_t)$. (E) Example result of tracking vesicle movements (shown as color-coded trajectories overlaid on the last frame of the time series) using a probabilistic method [69]. (F) Spatiotemporal rendering of the vesicle trajectories. (G) Comparisons have shown that the method correlates very well with manual tracking by expert human observers, suggesting that it may replace the latter, allowing experiments to be scaled up considerably.

knowledge of state changes) and (2) state update (using observation models based on a priori knowledge about the measurements given a particular system state). It can be computed analytically by means of Kalman filtering (in the case of linear system dynamics and Gaussian measurement noise) or to good approximation by means of sequential Monte Carlo schemes (in non-linear or non-Gaussian cases).

3.3. *Particle tracking in practice*

After particle tracking has completed, a host of kinetic and kinematic features can be readily computed from the obtained trajectories. Similar to cell tracking, obvious examples include total and net distances traveled, directional changes and persistence, instant and average velocities and accelerations, and histograms thereof to compare the distribution of these features between different populations. A more sophisticated and frequently studied feature in particle tracking experiments is the mean square displacement (MSD). It enables one to compute diffusion parameters of individual particles, as well as viscoelastic properties of the medium in which they move [80]. By varying the time-lag over which displacements are averaged in a trajectory, an MSD-time curve can be constructed, the shape of which is indicative of the mode of motion (Brownian, active transport, impeded, confined). The computations need to be carried out with care, however, as short trajectories and particle localization errors may give rise to apparent subdiffusion patterns [81], and the averaging process may conceal mode transitions within single trajectories [30].

Various examples in the recent literature illustrate the crucial role of automated particle tracking methods in discovering new biological phenomena. Computerized analysis of fluorescent speckle microscopy (FSM) movies of migrating cells have revealed that the protrusive behavior of the plasma membrane at the leading edge during migration is mediated by two spatially colocalized but kine(ma)tically and molecularly distinct actin networks related to the lamellipodia and the lamella, respectively, where the former

appear to serve an exploratory function while the latter are responsible for actual cell advancement [82]. Automated tracking and motion analysis of virus-like particles on the surface of tissue culture cells has provided new insights into the mechanisms involved in virus/receptor confinement before internalization [83]. Tracking of human immunodeficiency virus-1 (HIV-1) complexes have revealed directed movements characteristic of both microtubule- and actin-dependent transport within the cytoplasm towards the nuclear membrane, which contributes to the understanding of the cellular factors cooperating with or restricting HIV-1 infection [84]. As a final example, it has been shown by single-particle tracking that to a large extent, the mobility of protein assemblies within the cell nucleus correlates with the compaction of chromatin [85].

4. From cells to organisms

One of the major goals of biological research in our postgenomic era is to gain full understanding of the processes by which the genome directs the development of a single-cell zygote into a multicellular organism. Complete knowledge of the gene regulatory networks giving rise to specific phenotypes will dramatically advance the discovery of drugs and, ultimately, the development of clinical therapies. Model organisms such as the nematode worm *Caenorhabditis elegans* and the zebrafish are now widely used to study developmental phenomena efficiently. However, the imaging and quantification of every cell cleavage and every cell–cell interaction in an entire developing organism, as well as the phenotypical characteristics and behaviors of adult organisms, pose enormous challenges in terms of both image acquisition and image analysis [86–89]. Concerning the latter, it is safe to say that such studies constitute the ultimate test cases for automated tracking and motion analysis methods. Nevertheless, recent literature has shown first successes in the development and application of new methods for this purpose.

4.1. Tracking embryogenesis

The indispensability of computational tools for studying embryogenesis quantitatively has already been recognized for some time. Interactive computer-assisted systems facilitated the segmentation, reconstruction, visualization, and motion analysis of every cell and nucleus in a developing embryo [90,91], but required substantial manual effort in tracing and editing contours, limiting the practical use of these systems to the very early stages of embryogenesis. Automation of cell tracking in these stages is possible by applying basic image filtering techniques for cell segmentation and using spatial distance or the degree of cell overlap between image frames for cell association [86]. However, problems easily occur at later stages, due to the rapidly increasing number of cells and the cell density (Fig. 3).

In the endeavor to track embryogenesis much further down the line, alternative methods have been developed, based on fitting spherical models for segmentation and using heuristics about the

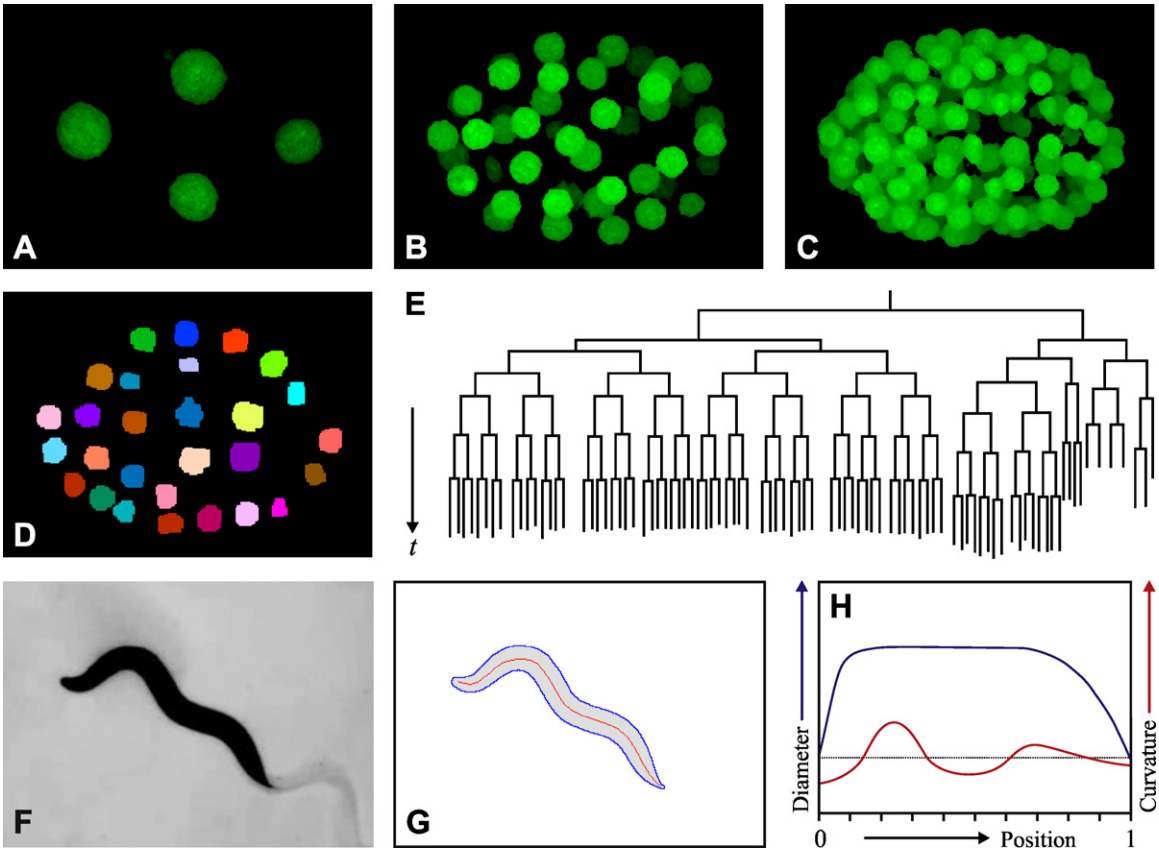

Fig. 3. Organism tracking. (A–C) Volume renderings of three time points (3D image stacks) from a time-lapse fluorescence microscopy data set, showing *C. elegans* embryogenesis from the four-cell stage to approximately 1 and 2 h later, respectively. The rapidly increasing number of cells and cell density pose a real challenge for automated cell tracking. (D) Result of 3D segmentation (only one plane is shown here) obtained by applying a level-set based model-evolution algorithm and morphological postprocessing to the stack rendered in (C). (E) Tracking results can be conveniently summarized and presented (after completion or even in the process) using lineage trees, which give a clear picture of mother–daughter relations of cells, division times, and symmetry breaking events. (F) Single frame from time-lapse image data acquired using a dissecting microscope and showing the morphological and behavioral phenotypes of an adult worm. (G) Owing to the high contrast in the image, the worm body can be accurately segmented using intensity thresholding, and simple morphological operations subsequently yield the outer contour and centerline ("skeleton"). (H) Finally, a variety of morphological features, such as body diameter and curvature, can be easily computed at any position along the extracted centerline.

shape and size of the nuclei at the various stages of the cell cycle to disambiguate potential matches between time frames [92,93]. The use of additional cues is of crucial importance when tracking densely packed cells through mitosis, when the telophase daughter nuclei are sent to the distal ends of the cell, and the two newborn cells may easily end up being closer to neighboring cells than to the mother cell in the previous frame. Several methods were recently developed for tracking and lineage construction of cultured cell populations [44,94] which may also prove useful for tracking embryogenesis. In line with the foregoing sections, especially the use of contour models, motion models, and probabilistic filtering schemes [44] can be expected to add considerably to the much-needed robustness of cell tracking for this application.

4.2. Tracking organism behavior

In order to get a full picture for genotype–phenotype mapping, quantitative studies of the development of an organism from the single cell stage to maturity must be complemented by analyses of the behavioral (ab)normalities of the adult subjects. This brings us to yet another (and in this article final) level of spatial and temporal observation, with its own peculiarities requiring special attention (Fig. 3). In the case of *C. elegans* tracking, on which we will focus in the sequel, a large (inter- and intra-subject) variability in appearance and behavior may be observed. For robust geometric modeling, it has been proposed [95] to make a distinction between the principal shape (the resting width-profile, length, and body orientation), and the conformation (bending patterns or curvature profiles) of the worms.

The use of standard stereo dissecting microscopes for worm tracking usually results in fairly high-contrast images, so that simple intensity thresholding can be used to segment the subjects from the background. Convenient centerline representations of the worms can then be easily obtained by applying "skeletonization" algorithms from mathematical morphology [96–98] or by using curve fitting procedures [95]. The segmentation and association tasks become more difficult, however, when studying multiple worms simultaneously for social behavior, where subject interactions, overlaps, or complex entanglements may occur. In addition, to be able to distinguish forward from backward movement, the head and tail need to be identified. Here too, similar to cell and particle tracking, the trend in the development of more robust tracking methods is in the direction of model-based and probabilistic estimation approaches [95].

4.3. Organism tracking in practice

For the presentation of the deluge of information resulting from exhaustive cell tracking during embryogenesis, cell lineage trees (Fig. 3) can be very helpful. Such trees not only offer a convenient visual impression of mother–daughter relations of cells, they also give a clear picture of division times, (a)synchronous divisions, symmetry breaking events, and even (by color coding) of gene expression levels, for which quantitative metrics can be computed straightforwardly. However, in order for lineage related analyses to make biological sense, the reconstructed trees need to be flawless. Since a single tracking error will invalidate the entire corresponding subtree, this imposes extremely high demands on the accuracy and robustness of cell tracking algorithms. Because even state-of-the-art algorithms are not quite error-free, careful manual curation and postediting of the resulting trees will always be necessary. Several software tools for visualizing, editing, and comparing cell lineage trees are publically available [99,100].

For the quantification of adult morphological phenotypes, geometrical features such as body length, area, thickness, symmetry, and curvature can be easily extracted upon successful segmentation [96]. As for behavioral phenotypes, *C. elegans* locomotion can be roughly classified into forward movement, backward movement, rest, and curl, the duration and change-rates of which are important parameters [98]. Automated tracking systems have been successfully used for quantifying a variety of morphological and behavioral patterns under controlled conditions (summarized in [96]). The statistical clustering of phenotypic patterns and their matching with genotypic classes and environmental conditions will yield new insights into the different mechanisms driving organism development and how to influence them beneficially.

5. Conclusions

In concluding this article, we summarize the most important observations and their implications for future research. First, in view of the data explosion that is currently taking place in cell and developmental biology, it is increasingly realized that powerful software tools are now essential on the road to discovery and breakthrough. The massive change in scale of biological investigations not only calls for efficient solutions for data management, but also requires computational methods for objective and reproducible processing, analysis, and interpretation of the data. While the first challenge has already resulted in open standards for storing and sharing image and meta data, the problem of how to best automate the actual "data crunching" is still being vigorously researched by many groups worldwide, and in this article we have attempted to provide the reader with a good overview of recent efforts in the field to develop methods for tracking and motion analysis of objects in time-lapse microscopy image data.

Second, the exponential growth of publications in the past few years on bioimage informatics related problems is not just a testimony of the utter need for and the development of computational tools, but also of the fact that the field is still very much in an exploratory phase. The general conclusion emerging from the current body of literature seems to be that there are no universal solutions to tracking problems in cell and developmental biology, and that tools pretending the existence of such solutions (such as many commercial software packages) generally show mediocre performance. This is understandable by realizing that, especially in developmental experiments, one must be concerned with the analysis of events at the molecular level (nanometer scale), the cellular level (micrometer scale), up to the organism level (millimeter scale), that is at least six orders of spatial (not to mention temporal) detail, each with its own idiosyncrasies. However, the advent of whole-body scanners for fluorescence and bioluminescence tomography imaging, and the desire to be able to quantify (sub)cellular processes within intact organisms, will push the demand for integrated methods capable of tracking motion at all relevant scales simultaneously. Currently, several trends can be observed in the development of new tracking methods, including the increasing use of models (of object shape and dynamics as well as image formation) and probabilistic (Bayesian) estimation methods, which are known from other fields to yield improved robustness.

Finally, since every specific tracking problem currently requires its own dedicated solutions to optimally extract and exploit the information contained in the data, investigators are constantly faced with the challenge to develop their own software tools. This is increasingly possible, even for users without expertise in computer programming, by means of commercial and open-source tools facilitating the assembly of existing image processing algorithms and the integration of custom-designed algorithms. However, biology has by now unquestionably developed into a multidisciplinary field, and it seems that the joint optimization of all aspects of biological experimentation (sample preparation, image acquisition,

image analysis, data modeling, and statistics) is best achieved by a close collaboration between biologists, chemists, physicists, mathematicians, statisticians, as well as computer scientists, all the way from experiment planning to the ultimate interpretation of the results. Academic environments and research programs fostering this collaboration will likely prove to be the main contributors to progress in biology.

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