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Automated Cell Tracking using Motion Prediction-based Matching and Event Handling

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Abstract

Automated cell segmentation and tracking enables the quantification of static and dynamic cell characteristics and is significant for disease diagnosis, treatment, drug development and other biomedical applications. This paper introduces a method for fully automated cell tracking, lineage construction, and quantification. Cell detection is performed in the joint spatio-temporal domain by a motion diffusion-based Partial Differential Equation (PDE) combined with energy minimizing active contours. In the tracking stage, we adopt a variational joint local-global optical flow technique to determine the motion vector field. We utilize the predicted cell motion jointly with spatial cell features to define a maximum likelihood criterion to find inter-frame cell correspondences assuming Markov dependency. We formulate cell tracking and cell event detection as a graph partitioning problem. We propose a solution obtained by minimization of a global cost function defined over the set of all cell tracks. We construct a cell lineage tree that represents the cell tracks and cell events. Finally, we compute morphological, motility, and diffusivity measures and validate cell tracking against manually generated reference standards. The automated tracking method applied to reference segmentation maps produces an average tracking accuracy score (*TRA*) of 99%, and the fully automated segmentation and tracking system produces average *TRA* of 89%.

Keywords

Cell tracking; cell event detection and handling; graph algorithms; motion analysis

1 INTRODUCTION

Studies of living organisms in biology and medicine require the analysis of their static and dynamic properties. Advances in imaging technologies have enabled the acquisition of time-lapse sequences of 2D and 3D data at the cellular and molecular level. Research in medicine and biology has become increasingly dependent on quantitative analysis of this type of imaging data [1] for extracting information about cell metabolism, growth, reaction to

stimuli, and reproduction [2]. The process of identification and tracking of each cell in an imaging sequence is necessary for measuring static and dynamic cell attributes.

Quantitative analysis includes the study of cell morphometry, such as the cells' shape and their dynamic characteristics in a sequence including the cell lifetime, motility, number of divisions, and morphological changes with time among other characteristics. The quantitative analysis of time-lapse microscopy images is a key for detection of disease patterns and can be used in decision making to make a reliable diagnosis. Research in pathology is a very significant step for diagnosis of a large number of diseases including most cancers. The ability to non-invasively track the delivery of various therapeutic cells (e.g. T-cells and stem cells) to the tumor site, and subsequent proliferation of these cells, would allow better understanding of the mechanisms of cancer development and intervention. Moreover, data collected from individual patients at the anatomical, cellular and molecular levels, offer unprecedented possibilities to design personalized therapies. Studies of disease mechanisms are valuable for clinical research areas such as stem cell research, tissue engineering, drug discovery, genomics, and proteomics. Morphological changes correlate with disease progression. Study of cell lineage relationships is significant for stem cell research, and disease etiology and progression studies [3], [4]. The statistical analysis of the tracking measurements will assist researchers to more precisely track the genetics of individual cells and detect and understand cell behavior and abnormalities. These applications depend on accurate cell tracking of individual cells that display various behaviors including mitosis, merging, rapid movement, and entering and leaving the field of view.

The data size of a single cell image sequence is typically in the range of hundreds of megabytes. Diagnosing a disease after manually analyzing numerous data requires intensive and laborious work and long time. Furthermore, manual analysis is dependent on the level of experience of the human operator, and is almost certainly non-reproducible. As a consequence, the use of automatic analysis and quantification can significantly improve the reproducibility, efficiency, and accuracy of cell studies, and overall benefit the patient.

Cell tracking methodologies can be divided into (i) tracking by model evolution and (ii) tracking by detection.

Model Evolution Methods –

These techniques integrate the stages of segmentation and tracking into a joint model that represents the cell regions and their evolution with time. In this framework topological changes may account for cell events. Model Evolution methods frequently utilize parametric or implicit active contour models. Active contour methods for image segmentation allow a contour to deform to partition an image into regions. Deformable models try to fit one frame and use the result as initialization in the evolution model of the next frame. The association step is implicitly solved and established. However these techniques may require topology constraints and are computationally demanding especially for sequences of high cell density.

Parametric active contour models use explicit representations of the cell contours and perform segmentation by energy functional minimization [5], [6]. The classic snake methods

cannot address topological changes related to cell events therefore they required some adjustments.

Implicit active contour models are often implemented with level sets [7], [8], [9]. The primary drawback, however, is that they are slow to compute. The level set can be initialized manually as an initial contour, or automatically by leveraging prior information about the shape and other characteristics of cells to track multiple cells [10]. Zhang et al [11] proposed coupled geometric active contours and used one level set function for each cell. The coupling constraints are applied to avoid cell overlapping. Dufour et al [12] extended this framework to 3D analysis.

Tracking by Detection –

These approaches typically detect the cells in each frame and then match cells between consecutive frames. The cell detection stage is achieved by image segmentation. The cell matching stage is completed by data association and cell correspondence techniques. Their main advantages of tracking by detection techniques are that they (i) can work on lower imaging frequencies than the model evolution techniques, (ii) involve advanced data association techniques for tracking, and (iii) separate the segmentation and tracking tasks, therefore different segmentation techniques can be used with a single tracking algorithm. Cell detection may be achieved by image segmentation techniques based on intensity, texture or gradient information [13], [14], [15], [16]. Feature-based segmentation techniques have been utilized for cell detection as described in [16], [17], [18]. Several deep learning approaches have also been proposed for cell detection, segmentation and classification in digital pathology and microscopy applications and have produced promising results [19], [20], [21]. However, most of the deep learning-based models do not implement a complete cell tracking system that requires cell matching and cell event handling stages.

Cell matching techniques often times use probabilistic functions between frames. Data association techniques that perform cell linking may be categorized into Bayesian and Non-Bayesian. Bayesian approaches compute the full distribution in Data Association space based on prior and posterior information. Non-Bayesian approaches find the Nearest Neighbor as the maximum likelihood estimate from all the Data Association possible solutions. Tracking by detection techniques may face challenges in mitosis tracking and when touching cells are segmented as a single cell. In previous reports in the literature, the authors in [22] used seeded watershed to address touching cells, followed by feature-based cell matching. They performed image-by-image track linking by means of integer programming. Yang et al [23] proposed to use watershed and mean shift to identify cell cycle progression but did not construct cell lineage.

In the tracking stage, when we encounter increasing cell density, the temporal association step requires sophisticated strategies to deal with one-to-many and many-to-one matching problems [8], [13], [14], [24]. In [13] image-by-image linking is accomplished using integer programming. Padfield et al [8] introduced a coupled minimum-cost flow algorithm for mitosis and merging events coupled with some operations on particular edges. They used linear programming to choose the edges of graphically represented constraints. In contrast to image-by-image linking techniques, batch algorithms use information from future frames or

the complete image sequence to create cell tracks and detect cell events [25], [26]. The authors in [27] proposed a batch algorithm that optimizes a probabilistic scoring function and may address false positives and false negatives in detection stage. It can also handle cell mitosis, apoptosis and other cell events.

Another subgroup of methods combines detection and data association using probabilistic prediction models. These methods predict global motion and characteristic parameters of the objects. Earlier approaches used Kalman filtering followed by Particle Filtering techniques [15], [28]. They use sets of random samples to describe the most likely states of a system. They use the temporal structure of distributions and determine the measurements using importance sampling. Particle filters work well for a small and fixed number of cells. In the approach proposed in [29], the authors used marker controlled watershed and region merging with context information for cell detection and delineation. They performed tracking using modified mean shift and Kalman filtering.

The aim of this work is to develop automated methods that successfully detect and track cells enabling the analysis of their static and dynamic behavior including cell morphology, cell migration, and changes in cell states (mitosis and apoptosis, for example). To reach these objectives we introduce automated techniques for cell detection, segmentation, tracking, construction of lineage trees of progenitor cells, and cell quantification. Overall one may argue that many approaches to cell tracking have been developed in the past, but most are focused on the same type of optical imaging techniques, require extensive post-processing, and are parameter intensive.

The tasks of automatic cell detection, segmentation, and tracking in time-lapse fluorescent microscopy images pose a difficult problem due to high variability in images because of numerous factors like differences in slide preparation (fluorescent concentration, presence of foreign artifacts, time to prepare the sample, and time between each time lapse image for the same sample, etc) and image acquisition (corruption by different types of noise, specific features of the microscope such as resolution and contrast limitations, etc). To be able to capture fluorescent microscopy images and observe live cell processes, like cell migration, apoptosis and proliferation, light exposure is kept minimal to reduce the photodamage, but this reduces the image quality [30], [31], [32].

We propose a fully automated tracking method to overcome these challenges and achieve high level of accuracy in these different cases. We outline the key contributions of this work next. (i) In the cell matching stage, we utilize the predicted cell motion in a probabilistic Maximum Likelihood Bayesian decision framework assuming Markov dependency to find cell correspondences between consecutive frames. (ii) In cell event detection, we find a solution that minimizes a global cost function, given cell neighborhood constraints, defined over the set of all cell tracks to perform track linking and to identify the cell states in the time-lapse sequence. Our system automatically constructs the lineages of proliferating migrating cells, which is a critical and required step for understanding cell behaviors. It produces measurements of static and dynamic cell attributes. The proposed system is applicable to varying cell shapes, types, and densities and image sequences of reduced

image quality. Overall, it enables accurate quantitative analysis of cell events, and provides a valuable tool for high-throughput biological studies.

In section 2, we present our proposed cell tracking method where we describe the pre-tracking stage, followed by an overview of the motion estimation and optical flow methods. We also introduce the combined local-global optical flow technique that we applied to our data, our likelihood-based bi-frame cell matching technique to link cells between frames, the proposed algorithms to create cell tracks and the acyclic graph, and cell event handling. In section 3, we show our cell tracking experiments and validation results and in section 4 we summarize the main findings of this work.

2 METHOD OVERVIEW

The goal of cell tracking is to identify all cells throughout the time-lapse sequence in order to follow their motion and detect the main events such as migration, mitosis, apoptosis, entering and leaving the field of view. Our method belongs to the category of tracking by detection. It uses cell indicator functions of each frame produced by our cell delineation technique to perform cell tracking and quantification. We propose a method for automated tracking of biological cells in time-lapse microscopy by motion prediction and minimization of a global probabilistic function for each set of cell tracks. We identify cell events by backtracking the cell track stack and forming new tracks to determine a partition of the complete track set.

As seen in Fig. 1, we first apply cell detection and delineation [33]. A pre-tracking stage is required to separate cell clusters to reduce under-segmentation, and to compute cell characteristics to be used for probabilistic cell matching and cell quantification. Then we estimate the cell motion by use of a variational multi-scale optical flow technique. Next, we apply the motion field to calculate warped cells and we apply a maximum likelihood decision approach on a probabilistic function to find cell correspondences. We then construct the cell linked lists to represent cell tracks and we backtrace the lists to detect overlapping tracks and identify and handle the cell events. After finding all cell events we construct the cell lineage tree that stores and visualizes the cell events. In addition, we calculate cell characteristics and their evolution with time to perform quantitative analysis and visualization.

We pursue a solution of the cell detection problem in the joint spatio-temporal domain to overcome weaknesses of previous works that operate only on the spatial domain. We employ a PDE-based formulation of spatio-temporal motion diffusion to detect the cell motion [34], [35]. We apply an intensity standardization technique to address intensity variability complicating frame-to-frame analysis in differential techniques. To refine cell delineation accuracy produced by motion diffusion-based segmentation, we use energy minimizing geometric active contours that assume a piece-wise constant image region model as a special case of the Mumford-Shah segmentation framework. Furthermore, we perform temporal linking of the region-based level sets to allow for faster convergence and to resolve non-convexity that affects energy-based minimization that is typical in image analysis inverse problems [33].

A tracking algorithm ideally should have the capability to address the main cell events that occur in a sequence. These events are (i) *Cell Mitosis: division of a cell into two daughters* (ii) *Cell Disappearance: cell leaving field of view or collision* (iii) *Cell Apoptosis/Necrosis: death of a cell* (iv) *New Entering Cell: cell entering the field of view*, and (v) *Cell Reappearance: cell re-entering the field of view*.

The association problem is usually the most complex and most challenging stage of the tracking system. By increasing the number of cells, the complexity of the linking problem increases exponentially. Changes in cell shape such as cell stretching may also cause false appearances due to cell detection discontinuities. Fig. 2a depicts a model of the cell events of migration, mitosis, disappearance and appearance. Fig. 2b displays a pair of consecutive frames selected from an image sequence dataset that we use for our experiments. Furthermore, Fig. 2b contains all the detectable cell events that may occur. Cell event detection usually follows cell tracking and requires analysis of cell tracks.

Our tracking system produces the following data and information: globally linked cell indicator maps, the cell lineage tree, and the tracking output measurements providing the birth and death information, the mitosis events, and quantitative cell measures.

2.1 Pre-tracking

Given the cell indicator maps of an image sequence, we apply a pre-tracking stage to reduce the false positives in cell detection and over- and under-segmentation of cells. We first apply a decision threshold to distinguish between cell and non-cell regions in the cell indicator map. This value is determined by the minimum cell area for an object to be considered as a cell or part of the background. This procedure aims to remove the non-cell detected objects before tracking in order to reduce the false positives.

On the other hand, in datasets of high cell density, the cells can be so close that they can be falsely detected and segmented as a single cell leading to false negatives as shown in Fig. 3. In this case, we split clusters of multiple cells using geometrical shape priors, distance transform on the watershed relief, and the H-minima transform [36].

To separate fused cells, we apply watershed segmentation to the signed Euclidean distance map D , computed on the cell segmentation map B , so that $D = DT(B)$, where DT denotes the signed distance transform. This operation creates a boundary between the different cells in the cluster. The watershed transform has been proposed by many authors for nuclei segmentation [37], [38]. However, the number of peaks in the distance map may result in over-segmentation by splitting the clusters of cells into more regions than necessary. To reduce the number of over-segmented cell fragments and reduce spurious local minima, we use a morphological reconstruction technique that is the H-minima transform. H-minima Transform [36], [39] is a morphological operation obtained by erosion of the image D increased by a height h defined by

$$H(D, h) = R^e(D + h) \quad (1)$$

where R^ϵ is defined as the reconstruction by erosion morphological operator and h is a depth parameter. H-minima transform removes spurious local minima and avoids over-segmentation by suppressing all the regional minima whose depth is smaller than h . H-minima/maxima transform has been largely used in nuclei detection in biomedical images [40], [41].

As an example, we show in Fig. 4 a binary cell mask B , the cell label map L , the cell boundaries before cell separation, the distance map D , the watershed map obtained from the reconstructed distance map after H-minima transform H , and finally the corresponding watershed regions depicting the separated cells.

2.2 Estimating Cell Motion

We estimate the motion of each cell in a specific frame and predict the cell locations in the next frame prior to cell matching. This is a challenging task mainly because of potential limitations in temporal resolution, and variable types of cell motion. For example, the biological cells may follow a Brownian movement, which makes the motion estimation very hard. Next, we outline the principles and limitations of conventional optical flow computation and describe the combined local/global optical flow (CLGOF) technique that we utilize to address limitations of the conventional techniques.

2.2.1 Optical Flow Computation—The optical flow estimates the velocity of each pixel between two consecutive frames at times t and $t + \Delta t$ based on spatio-temporal image intensity variations. This method is used in computer vision to characterize and quantify the motion of objects.

Methods for optical flow estimation are based on the computation of partial derivatives of the image intensities signal. Two fundamental techniques were proposed in [42] and [43]. Lucas and Kanade [42] proposed a local method that uses a spatial constancy assumption. The method by Horn and Schunk [43] is a global method that supplements the optical flow constraint with a regularizing smoothness term.

In the computation of optical flow we usually make implicit or explicit assumptions that set constraints to the problem. We assume gradual changes of image motion of an object. That is, the image motion slowly changes in time. In practice, this means the temporal increments are fast enough compared to the motion of objects in a frame. In this case, the temporal difference approximates the derivative of the intensity with respect to time. An additional constraint is that of spatial smoothness that requires neighboring pixels to have approximately the same motion. Finally, we may assume that the gradient of the image intensities is not changed by the displacement.

Local optical flow estimation techniques enable to overcome the aperture problem, where Lucas and Kanade assumed that the optical flow is constant within a neighborhood determined by an integration scale ρ . The integration scale is produced by convolution with a Gaussian kernel K_ρ with standard deviation equal to ρ . They proposed to use a least square fitting method to solve the linear system in equation (2) and estimate the optical flow components $(u, v)^T$ [42]:

$$E_{LK} = \begin{bmatrix} K_\rho \star (I_x^2) & K_\rho \star (I_x I_y) \\ K_\rho \star (I_x I_y) & K_\rho \star (I_y^2) \end{bmatrix} \begin{bmatrix} u \\ v \end{bmatrix} = \begin{bmatrix} -K_\rho \star (I_x I_t) \\ -K_\rho \star (I_y I_t) \end{bmatrix}, \quad (2)$$

where I_x , I_y , I_t denote the pixel intensity derivatives along the x-, y- and temporal directions respectively. Because we cannot find solutions at all points, the resulting field is non-dense. Therefore an interpolation step is used to alleviate this shortcoming.

Another category of approaches perform global estimation to produce a dense flow field by minimizing a global functional with regularization constraints. Horn and Schunk [43] proposed to find the field $(u, v)^T$ as the minimizer of the following functional:

$$E_{HS} = \int_{\Omega} \left[(I_x u + I_y v + I_t)^2 + \lambda (|\nabla u|^2 + |\nabla v|^2) \right] dx dy, \quad (3)$$

where λ is a Lagrangian multiplier for imposing smoothness constraints to the optical flow field.

The solution of these diffusion-reaction equations is unique. Moreover, at locations where $|\nabla I| \approx 0$, the local flow cannot be computed but the regularization term provides an estimate based on neighboring pixels. Therefore this technique yields a flow estimate for the complete image domain and no interpolation is needed. However global differential methods may be more sensitive to noise than the local techniques. Flow fields are less regularized at noisy image regions because noisy regions are characterized by high gradients that overcome the smoothness regularization term.

2.2.2 Combined Local/Global Optical Flow Method (CLGOF)—The estimated vector fields of the local techniques may not be dense or may have many discontinuities [42], [44], while the global techniques solutions may not be robust [43], [44]. To overcome these drawbacks, we adopt a solution that combines the local and global optical flow estimation principles [44], [45].

The energy functional includes data and smoothness terms. The data term measures the deviations from the intensity constancy assumption and the gradient constancy assumption and is given by:

$$E_{Data}(u, v) = \int_{\Omega} \left(|I(\vec{\omega} + \vec{\delta}) - I(\vec{\omega})|^2 + \gamma |\nabla I(\vec{\omega} + \vec{\delta}) - \nabla I(\vec{\omega})|^2 \right) d\vec{\omega} \quad (4)$$

where $\vec{\omega} = (x, y, t)^T$, $\vec{\delta} = (u, v, 1)^T$, and γ denotes a parameter that regulates the effect of each deviation on the data term. A function $\Psi(s^2)$ may be applied to the integrand to moderate the effect of outliers. We adopt the approach in [44] that utilizes the form of $\Psi(s^2) = \sqrt{s^2 + \epsilon^2}$ with $\epsilon = 0.001$. The data term expression becomes:

$$E_{Data}(u, v) = \int_{\Omega} \Psi \left(|I(\vec{\omega} + \vec{\delta}) - I(\vec{\omega})|^2 + \gamma |\nabla I(\vec{\omega} + \vec{\delta}) - \nabla I(\vec{\omega})|^2 \right) d\vec{\omega}. \quad (5)$$

The purpose of the smoothness term is to penalize discontinuities of the optical flow field and is given by:

$$E_{Smooth}(u, v) = \int_{\Omega} \Psi \left(|\nabla_{ST} u|^2 + |\nabla_{ST} v|^2 \right) d\vec{\omega}, \quad (6)$$

where $\nabla_{ST} = (\partial_x, \partial_y, \partial_t)^T$ is the spatiotemporal gradient.

The joint energy functional is:

$$E_{CLGOF} = E_{Data} + \lambda E_{Smooth}. \quad (7)$$

Based on the calculus of variations, the minimizer of equation (7) is a solution of the Euler-Lagrange equations:

$$\begin{aligned} & \Psi' (I_x^2 + \gamma(I_{xz}^2 + I_{yz}^2)) \cdot (I_x I_z + \gamma(I_{xx} I_{xz} + I_{xy} I_{yz})) - \alpha \nabla \\ & \cdot (\Psi' (|\nabla_{ST} u|^2 + |\nabla_{ST} v|^2) \nabla_{ST} v) = 0 \\ & \Psi' (I_z^2 + \gamma(I_{xz}^2 + I_{yz}^2)) \cdot (I_y I_z + \gamma(I_{yy} I_{yz} + I_{xy} I_{xz})) - \alpha \nabla \\ & \cdot (\Psi' (|\nabla_{ST} u|^2 + |\nabla_{ST} v|^2) \nabla_{ST} u) = 0. \end{aligned} \quad (8)$$

$$\begin{aligned} \text{where } I_x &= \frac{\partial I(\vec{\omega} + \vec{\delta})}{\partial x}, I_y = \frac{\partial I(\vec{\omega} + \vec{\delta})}{\partial y}, I_z = I(\vec{\omega} + \vec{\delta}) - I(\vec{\omega}), I_{xx} = \frac{\partial^2 I(\vec{\omega} + \vec{\delta})}{\partial x^2}, \\ I_{xy} &= \frac{\partial^2 I(\vec{\omega} + \vec{\delta})}{\partial x \partial y}, I_{yy} = \frac{\partial^2 I(\vec{\omega} + \vec{\delta})}{\partial y^2}, I_{xz} = \frac{\partial I(\vec{\omega} + \vec{\delta})}{\partial x} - \frac{\partial I(\vec{\omega})}{\partial x}, I_{yz} = \frac{\partial I(\vec{\omega} + \vec{\delta})}{\partial y} - \frac{\partial I(\vec{\omega})}{\partial y}. \end{aligned}$$

Reflecting boundary conditions are utilized to solve the system of equations in (8).

This technique yields an approximation of the displacement field $\hat{\delta}$:

$$\hat{\delta} = \arg \min_{\vec{\delta}} E_{CLGOF}. \quad (9)$$

The displacement field solution is first estimated on a coarse grid of the image plane produced by downsampling, and then on the original grid. This multi-scale approach yields a more accurate global solution than a single-scale approach because it reduces false convergence to local minima of the energy functional. The final solution found at the coarse scale is used to initialize the finer scale [45]. An example of the optical flow estimate during cell division using this method is displayed in Fig. 5.

2.3 Applying Motion Field to Previous Cell Indicator Frame

The estimated displacement field $\hat{\delta}$ is used to calculate a warped frame $\hat{I}$ by:

$$\hat{I}(\vec{\omega} + \hat{\delta}, t + 1) = I(\vec{\omega}, t). \quad (10)$$

We define a cell indicator function $L: \Omega \rightarrow \mathbb{Z}$ that maps pixel values onto unique cell identifiers produced by the cell segmentation stage. We apply warping to the cell indicator frame to find $\hat{L}$ before linking the current and previous frame:

$$\hat{L}(\vec{\omega} + \hat{\delta}, t + 1) = L(\vec{\omega}, t). \quad (11)$$

The cell matching and linking stage is applied between the pairs of frames $\{I(\vec{\omega}, t + 1), \hat{I}(\vec{\omega}, t + 1)\}$ and $\{L(\vec{\omega}, t + 1), \hat{L}(\vec{\omega}, t + 1)\}$ using probabilistic decision functions. An example of the optical flow calculation and application for warping is displayed in Fig. 6.

2.4 Likelihood-based Bi-frame Cell Matching

The problem of finding the association with the highest probability is performed by computing the max-likelihood matching for each cell of the current frame among all the cells of the previous warped frame. We formulate bi-frame cell matching as a classification problem.

Let $\Xi_t = \{\xi_1^t, \xi_2^t, \dots, \xi_n^t\}$ be the states of a system with n cells at time t , $X_t = \{x_1^t, x_2^t, \dots, x_n^t\}$ be the set of observations for all cells at time t , and $S_t = \{s_1^t, s_2^t, \dots, s_n^t\}$ be the set of samples for all cells at time t . We also assume a Markov process such that the transition probability depends only on the state of the mother cell or the same cell in previous frame $P(S_{t+1}|S_t)$. We compute the likelihood that the cell i in frame $t + 1$ is connected with cell j in frame t . Assuming that each cell i in frame t corresponds to a state of nature s_i^t , we calculate:

$$\pi_{ij}^{t+1} = p(x_i^{t+1} | \xi_i^{t+1} = s_j^t). \quad (12)$$

Here we define a likelihood function π_{ij}^t based on the observations derived from the computed cell features. We currently use spatial proximity between cells of warped indicator function $\hat{L}(\omega + \hat{\delta}, t + 1)$ and cells of the current indicator function $L(\omega, t)$ to form the observation vectors x_j^t, x_i^{t+1} . Hence

$$\begin{aligned} & p(x_i^{t+1} | \xi_i^{t+1} = s_j^t) \\ & \propto \exp\left\{-(1/2)(x_i^{t+1} - x_j^t)^T \Sigma^{-1} (x_i^{t+1} - x_j^t)\right\}. \end{aligned} \quad (13)$$

We make a decision using a maximum-a-posteriori (MAP) rule that becomes a maximum likelihood (ML) rule assuming equal priors. We also use a reject option to model cell appearance when the maximum likelihood is still very low. Fig. 7 displays the cell matching procedure including cases of cell appearance, disappearance, and migration.

$$\hat{j} = \arg \max_j \pi_{ij}^{t+1}. \quad (14)$$

After making a decision we assign the predicted cell class $\hat{j}$ to the cell indicator map $L(\vec{\omega}, t + 1)$.

2.5 Creating Cell Tracks and Acyclic Graph

Overall, the tracking and cell detection problem can be viewed as a partitioning problem by cost minimization. We first introduce the framework for cell track representation, construction, and optimization.

Definition 2.1 (Cell track set). Let N be the number of frames in a time-lapse sequence, and M be the total number of cell tracks. We define the global set of cell labels among all the frames as $L_G = \{\xi_i^t\}$ and the set of all cell tracks in a sequence as Φ^M , such that $|\Phi^M| = M$.

Definition 2.2 (Individual cell track). We define each track $\phi_i \in \Phi^M$ as the set of identified cell states in the sequence.

$$\phi_i = \{\xi_i^t \mid t = t_{start}, \dots, t_{end}\}, \quad (15)$$

where t_{start} and t_{end} denote the first and last frame of the track in the sequence with $0 \leq t_{start} \leq t_{end} \leq N - 1$.

Cell tracking optimization problem —Our goal is to identify all cell tracks Φ^M in the sequence. This can be formulated as follows: Let $\mathcal{E}\{\Phi^M\}$ be the cost computed over a specific track set Φ^M .

Find cell track set

$$\hat{\Phi}^M = \arg \min_{\Phi^M} \mathcal{E}\{\Phi^M\}, \quad (16)$$

with the conditions

$$\Phi^M = \bigcup_{i=1 \dots M} \phi_i \quad (17)$$

$$\phi_j \cap \phi_k = \emptyset, \forall j \neq k. \quad (18)$$

After finding a solution $\hat{\Phi}^M$, we assign a track label $I \in L_G$ to each cell. The track duration is defined from the discrete time point of cell appearance till its disappearance, division, or reaching the end of sequence. If a new cell appears, then we create a new track. In the case of cell division we end the track of the mother cell and create two new daughter tracks.

2.5.1 Creating Cell Tracks—First, we create linked lists of the cell states that map each cell to its best match. This is accomplished by pairing each cell of the warped label map at time t with one cell in the label map at time t_p using the maximum likelihood approach described in Section 2.4.

Next, we use the created linked lists to construct the cell tracks. We traverse the set of cell states in reverse chronological order and we store the cell track information in a cell track array structure. The elements of this structure are quadruples $Q = [t, \xi_m^t, t_p, \xi_n^{t_p}]$, that contain the frame id t , cell label (indicator) id ξ_m^t , previous frame id t_p , and previous label id $\xi_n^{t_p}$. This procedure is outlined in Algorithm 1.

The previous procedure produces a cell track structure that holds the tracks ϕ_i and addresses cell appearance and disappearance. However, at the end of this stage some tracks may be partially overlapping that is in the case of cell division, where different cells have a common ancestor. We address these cases by finding the overlapping parts of two tracks ϕ_j and ϕ_k , and creating three new tracks, one for the parent $\phi_p = \phi_j \cap \phi_k$, and the 2 daughters $\phi_q = \phi_j - (\phi_j \cap \phi_k)$ and $\phi_r = \phi_k - (\phi_j \cap \phi_k)$. This procedure is outlined in Algorithm 2.

Algorithm 1

Construct cell tracks

Require: *Cell_Linked_Lists*

```

1: for each linked list in Cell_Linked_Lists from end to beginning of list do
2:   for each cell  $\xi_i^t$  in linked list do
3:     if  $\xi_i^t \notin \phi_k, k = 1, \dots, m$  previous track then
4:       set  $m \leftarrow m + 1$ , create a new track  $\phi_m$  in Cell_Track_Structure
5:       repeat
6:         find matching cell label  $\hat{j} = \arg \max_j \pi_{ij}^{t+1}$  in previous frame recursively in Cell_Linked_Lists
7:         add  $Q = [t, \xi_m^t, t_p, \xi_n^{t_p}]$  to Cell_Track_Structure
8:       until end of current linked list
9:     end if
10:  end for
11: end for
12: return

```

Algorithm 2

Identify and handle cell events

Require:

```

1: for each track  $\phi_i \in \Phi^M$  do
2:   while there exists a previous match  $s_j^{t-1}$  of  $\xi_i^t$  such that  $\hat{j} = \arg \max_j \pi_{ij}^{t+1}$  do
3:     search for  $s_j^{t-1}$  in Cell_Track_Structure and find matches
4:     if new match is found then
5:       if  $s_j^{t-1} \in \phi_k, k = i + 1, \dots, M$  then

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6:      {division is detected; form three new tracks; parent  $\phi_p$  and 2 daughters  $\phi_p, \phi_r$ }
7:       $\phi_p = \phi_i \cap \phi_k$ ;
8:       $\phi_q = \phi_i - (\phi_i \cap \phi_k)$ ;
       $\phi_r = \phi_k - (\phi_i \cap \phi_k)$ 
9:      {update cell track set}
       $\Phi^{M+1} \leftarrow (\Phi^M - \{\phi_i, \phi_k\}) \cup \{\phi_p, \phi_q, \phi_r\}$ 
10:     else if cell is only in previous tracks then
11:         close track  $\phi_i$  and set parent label to 0
12:     end if
13: else
14:     {migration is detected}
    add cell to existing track;  $\phi_i \leftarrow \phi_i \cup \{\xi_j^t - 1\}$ .
15: end if
16: end while
17: end for
18: return

```

2.5.2 Minimal Cost Cell Labeling—The individual maximum likelihood tracks ϕ_i constitute minimum error solutions according to Bayesian theory [46]. Therefore, the proposed algorithm can be considered as a forest of minimal cost chains with temporal constraints that minimizes the cost $\mathcal{C}$ in the universe of cell tracks Φ defined as:

$$\mathcal{C} = \sum_{\phi_i \in \Phi} C(\phi_i), \quad (19)$$

where $C(\phi_i)$ is the total cost of the cell track ϕ_i .

$$C(\phi_i) = \sum_{j \in \phi_i} c(\pi_{ij}), \quad (20)$$

where $c: \mathbb{R} \rightarrow \mathbb{R}$ is a decreasing function of sigmoid or exponential form. Our approach outlined in Algorithm 1 determines a greedy solution to this combinatorial optimization problem. The cell event analysis described in Algorithm 2 ensures that the solution set is non-overlapping and exhausts the universe of cell tracks for each sequence.

2.5.3. Representing Cell Tracks using an Acyclic Oriented Graph—Cell tracking results can be represented using an acyclic oriented graph. The nodes of such a graph correspond to the detected cells, whereas its edges coincide with temporal relations between them. The acyclic graph $G = (V, E)$ consists of a vertex set V and an edge set E such that $E \subset V \times V$. The condition for an ordered vertex pair $E_{i,j}^{t_1, t_2}(\xi_i^{t_1}, \xi_j^{t_2})$ to belong to the edge set E is:

$$E_{i,j}^{t_1, t_2}(\xi_i^{t_1}, \xi_j^{t_2}) \in E \Leftrightarrow (i = j \wedge t_2 = t_1 + 1) \vee (i \neq j \wedge t_1 < t_2 \wedge P(\xi_j^{t_2}) = \xi_i^{t_1}). \quad (21)$$

The function $P: \Xi \rightarrow \Xi$, where Ξ is the universe of cell states ξ_i , returns the parent ξ_a of an entity ξ_i that is $\xi_a = P(\xi_i)$. The first case of an edge in equation (21) represents cell migration, while the second case represents cell division. The graph is guaranteed to be acyclic because the edges are oriented and they follow the ascending temporal ordering of the cell state indicators within and between tracks.

We create an acyclic graph table by traversing the processed cell trajectories that were formed by the Algorithm 2 to find last frame, first frame, and parent track id. Finally we create an adjacency list of the graph nodes and sort and relabel the nodes to create a tree representation. We write the adjacency list to file and plot the constructed acyclic graph using node color coding that indicates the cell states of appearance, migration, division, and disappearance as displayed in Fig. 9. Finally, we assign graph node labels $L_G(V)$ to cells in each indicator image and create a global indicator function $F_L: \Omega \rightarrow L_G$ represented by a 2D+t label structure.

3 CELL TRACKING EXPERIMENTS

In our work, cells are first detected and delineated in all the frames of the sequence independently from the tracking method. The tracking is done sequentially throughout the whole time-lapse sequence, and each cell of each frame is paired to none, one or two cells of the next frame. We track the cells by associating the segmented cell regions and making connections to accurately handle physiological cell events that take place during the course of the imaging experiment.

In this section, we describe the cell tracking experiments including the validation against reference data for tracking and we discuss the results. In addition, we display and discuss the cell lineage trees, the detected cell trajectories, and cell quantification results.

The ground truth data for tracking consist of images with markers and a directed acyclic graph for each image sequence. To evaluate tracking accuracy we utilize a graph matching cost function that we describe next.

3.1 Tracking Evaluation

The tracking accuracy measure symbolized by TRA aims to evaluate the capability of an automated algorithm to detect and track cells versus reference tracking data that were described in [18]. TRA calculates the difference between the acyclic oriented graph produced by the tested method and the TRA-GT-F reference graph [47]. TRA calculates the least number of graph operations needed in order to transform the test graph produced by the tested method to TRA-GT-F.

The TRA measure is given by

$$TRA = 1 - \frac{\min(TRA_P, TRA_E)}{TRA_E}. \quad (22)$$

In this equation, TRA_P is the weighted sum of the graph matching operations of node splitting, node adding, node deletion, edge deletion, edge addition and edge semantics

editing, TRA_E denotes the cost of constructing the reference graph from the beginning. TRA_E is given by

$$TRA_E = w_{FN}|M| + w_{EA}|E|. \quad (23)$$

In this equation, $|M|$ denotes the cardinality of the node set, and $|E|$ denotes the cardinality of the link set of TRA-GT-F. The division operation in equation (22) normalizes the graph cost, and the subtraction from 1 defines an evaluation function that increases with better tracking, such that $TRA \in [0, 1]$. Because the acyclic oriented graphs represent the cells, their states, and the associated cell events, TRA also quantifies the capability of an algorithm for automated cell event detection.

3.2 Automated Cell Tracking Results

The objective of cell tracking is to identify and follow the segmented cells in a time-lapse sequence. We performed cell tracking experiments and validated them against reference tracking results using the TRA measure. We then computed and visualized the cell lineage trees produced by the automated tracking algorithm. We finally computed static and dynamic cell measures.

We validated our tracking approach in two stages; first we used cell segmentation maps that were manually generated by the organizers of the IEEE ISBI Cell Tracking Challenge that we denote by CTC [48]. Then, we tested our fully automated cell segmentation and tracking system that includes our cell segmentation method denoted by ST-Diff-TCV [33].

3.2.1 Experiment 1: Tracking validation using reference segmentation—Table 1 lists the tracking accuracy measure denoted by TRA_{GT} and calculated by equation (22) using as input the manual cell segmentation masks denoted by GT for the ground truth. In Table 1, we observe that the tracking method produces very high accuracy rates with an average of 0.992. These results show that our tracking approach is able to detect cell events very efficiently given a reference segmentation. It was able to detect cell migration, mitosis, appearance and disappearance events that occur in the test sequences. We note that when we use the reference label maps, the tracking performance level is about the same for simulated and real sequences.

Even when reference segmentation is given, tracking by itself presents certain challenges. Microscopy live cell image sequences may have temporal and spatial resolution limitations and cell may move at variable speeds thus complicating the motion analysis and matching stages. Computing the displacement field between each two pairs of frames using the *CLGOF* method before our bi-frame matching, enables us to successfully overcome the problem of large time and space discrepancies for a robust and efficient cell tracking system.

3.2.2 Experiment 2: Tracking validation of fully automated tracking methodology—Next, we validated our joint automated segmentation and tracking methodology that employs our ST-Diff-TCV segmentation method [33] to delineate the cells prior to tracking. We denote our fully automated tracking method by ST-Diff-TCV-Tr. Table 2 displays the tracking validation results as TRA values along with the segmentation

accuracy expressed by Dice Similarity Coefficient (*DSC*) values. Overall, our method produces very promising tracking rates with an average tracking rate *TRA* of 0.891. We note that the segmentation stage, which yields average *DSC* of 0.893, allows for efficient tracking detection. In specific cases, over- or under-segmentation of a cell may significantly reduce the tracking accuracy. The lower *TRA* value for the C2DL-MS02 sequence is caused by the very low contrast-to-noise-ratio and signal-to-noise-ratio characteristics of this sequence and very elongated cell morphology with high intensity variability inside the cell body. These effects result in over-segmentation, which creates false positives in cell detection and tracking. In addition, under-segmentation produces false negatives in cell detection that are propagated to the tracking stage. In the N2DL-HeLa sequences some clustered cells were not delineated correctly, resulting in under-segmentation that reduced the tracking accuracy. Cell segmentation errors also occurred in C2DI-MS02 sequences because the cells have elongated shapes and variable levels of intensity in different parts of each cell resulting also in low contrast. On the other hand, ST-Diff-TCV-Tr tracking performed very well for N2DH-GOWT sequences even though they include several challenges such as heterogeneous average cell intensities and unlabeled cell nucleoli. Our results support the premise that tracking is dependent on segmentation.

3.2.3 Comparisons with Other Methods—Here we compare the performance of our fully automated tracking method ST-Diff-TCV-Tr with cell tracking methods developed by research groups that participated in previous Cell Tracking Challenges and were published in [18], [47]. In our comparisons we included methods that produced the top 3 overall tracking measures over our test data in CTC. These methods are denoted by KTH-SE, IMCB-SG_1, and UP-PT. KTH-SE includes 3 algorithms using bandpass [18], variance, or ridge segmentation and performs track linking based on the Vitterbi algorithm [27]. IMCB-SG_1 finds seeds using the extended maxima transform and applies seed-controlled watershed for cell segmentation. Tracking is accomplished by linking seeds between frames. UP-PT uses a local interest point detector for cell detection and applies Euclidean distance-based nearest neighbor search to SIFT descriptors for tracking [17]. Furthermore, we included results from the methods FR-Ro-GE and HD-Har-GE that have produced top ranked *TRA* values for specific sequences as described in [47]. FR-Ro-GE uses convolutional neural networks for segmentation [19] and greedy label propagation for tracking. The authors proposed a fully convolutional network (fCNN) architecture that they denoted as the U-net that includes an upsampling stage. The utilization of deep learning technique implies very good segmentation results, however tracking was not successful for the N2DH-GOWT1 sequences. HD-Har-GE utilizes thresholding and watershed transformation for segmentation and local spatio-temporal optimization for tracking [49]. We also included the average performance by the top 3 methods of CTC KTH-SE, IMCB-SG_1, and UP-PT denoted by CTC_T3, and the average performance of all 21 participants of CTC (denoted by CTC_ALL). The latter measure may be used to compare the performance of individual methods to the average level of competition in the CTC challenge.

Table 3 contains the *TRA* scores for all methods over each subset of the tested image sequences. We observe that our method ST-Diff-TCV-Tr yields significantly better tracking rates than CTC_ALL for each sequence. Also, it produces higher *TRA* scores than the

average *TRA* of the top 3 methods -denoted by CTC_T3. When compared to individual methods, ST-Diff-TCV-Tr produces higher TRA rates than 4 of the 5 methods that had stood out in CTC challenges.

In Fig. 8 we display the summarized TRA rates for each of the compared methods over all the datasets. We note that the average tracking performance of our method is competitive with the top 3 methods from CTC. These results show that our method produces very good levels of accuracy for each set and on average. While the KTH-SE method is the only method that yields higher average tracking performance than ST-Diff-TCV-Tr, it requires selection of segmentation algorithm for each sequence [47], therefore it may not be considered as a fully automated method and may not be directly comparable with the other methods.

We also performed paired t-tests between the TRA scores of ST-Diff-TCV-Tr and the scores produced by each one of the other methods. The test versus CTC_ALL produced a p value of 4.3×10^{-5} indicating that the tracking performance improvement of ST-Diff-TCV-Tr versus CTC_ALL is statistically significant. All other p-values were greater than 0.05.

3.3 Visualization of Cell Trajectories

The tracking accuracy measure *TRA* that was defined in equation (22), computes the similarity between lineage trees identified by our methodology versus reference lineage trees that were constructed manually by human operators.

We illustrate in Fig. 9 the cell lineage trees automatically generated by our tracking approach using the reference cell identifier maps (left) and our automated cell segmentation results (right) for a simulated sequence that includes numerous mitosis events. The two trees in this example have very similar structure that implies high *TRA* for both methods. Indeed, $TRA_{GT} = 0.997$ and $TRA = 0.973$. Furthermore, Fig. 10 displays the cell lineage tree produced by our fully automated cell segmentation and tracking methodology on a dense real test sequence of HeLa cells. A cell lineage tree in general corresponds to the directed acyclic graph. It represents and visualizes the tracked cells and the cell events detected by our tracking methodology. More specifically, our automatically constructed cell lineage trees $CLT(V, E)$ consist of nodes V that represent the cells that are identified across the sequence, and links E that represent the cell event evolution, namely cell migration, cell appearance and disappearance, and cell mitosis. The dashed links denote cell migration and the continuous links denote a parent-daughter relationship.

As an application of cell tracking, we generated cell trajectories in the projected 2D domain in Fig. 11a and in the 2D+t domain displayed in Fig. 11b. The system produces automatically the cell trajectory graphs using the global cell indicator functions. In these figures the trajectories are color-coded and each color represents the biological events during the lifetime of a single cell. The track IDs are also displayed next to the end of each cell trajectory for reference. The cell trajectories can effectively provide a visual analysis tool of a biological experiment. More specifically, they reveal mother and daughter relations, and metrics such as symmetry and division times can be extracted from cell lineage. Types of motion can also be visually inferred from the 2D projections of trajectories. Straightforward

applications include the development of predictive models for stem cell population growth and design, and optimization of subcultural strategies.

3.4 Cell Quantification

A significant application of tracking is cell quantification and quantitative analysis. Quantification is the computation of biologically meaningful cell measures that can be divided into morphological, motility, diffusivity, and velocity measures. Morphological measures include the area, perimeter, and shape features such as the major and minor principal axes geometry, eccentricity, solidity, and convexity. More sophisticated shape features can be computed using Fourier descriptors, Independent Component Analysis (ICA), and Principal Component Analysis (PCA). Motility measures are computed from the trajectories of the tracked cells using piecewise linear approximation. Typical motility measures include the total distance traveled by each cell, the net distance that is the distance between the start and the end points in a trajectory, the total trajectory time, or cell life time. An advanced measure of diffusivity is the Mean Squared Displacement (MSD) that is computed by the second-order moment of displacement as a function of time point difference and is defined as follows

$$MSD(n) = \frac{1}{N-n} \sum_{i=1}^{N-n} d^2(\omega_i, \omega_{i+n}), \quad (24)$$

where $\omega_i = (x_i, y_i)$ is the centroid of a cell at time point i , N is the total trajectory lifetime, and n is the interval for computation of MSD. A frequent selection for the distance function $d(\cdot, \cdot)$ is the Euclidean distance $d(\omega_i, \omega_{i+n}) = \|\omega_i - \omega_{i+n}\|_2$. MSD is used for characterizing the mode of cell motion. We can accomplish this by observing the MSD-time curve. Some identifiable modes of cell motion are Brownian, anomalous diffusion, region-confined motion, directed motion, or immobility.

In our method we compute 26 morphological, motility, and diffusivity measures. Fig. 12 illustrates a visualization and quantification example for the sequence Fluo-C2DL-MS02. Fig. 12a and Fig. 12c display the detected cell trajectories in the projected 2D domain and the 2D+t domain respectively. Fig. 12b and Fig. 12d illustrate the MSD function versus time and the mean MSD value for each cell. In these plots we can identify different motion modes including linear and exponential types. A linear MSD type indicates Brownian motion. A MSD curve approximated by a power law function may indicate superdiffusion, normal diffusion or subdiffusion, depending on the approximated power coefficient value. Our system automatically produces the quantification measures and related plots for all cells and trajectories for an input time-lapse sequence.

4 CONCLUSION

Our work concentrated on the design, development and validation of an automated cell tracking system. We propose a formulation of cell tracking as an optimization problem of graph partitioning and we introduce a solution in this context. We utilize the predicted cell motion by the multiscale Combined Local-Global Optical Flow technique in a probabilistic decision framework to find inter-frame cell correspondences. This increases the robustness

of tracking with respect to temporal sampling limitations of the image sequences and diverse cell motion types. Another contribution of this work is the fully automated cell tracking, lineage construction and quantification system that enables the analysis of very large volumes of data.

We performed extensive experiments for evaluating the performance of our method. Our tracking and event detection technique when applied to reference segmentation maps produces an average *TRA* measure of 99%, while our fully automated segmentation and tracking method denoted by ST-Diff-TCV-Tr performed at 89%. We performed comparisons to leading-edge cell tracking methods, which indicate that the proposed technique produces very good tracking accuracy relatively to these techniques.

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Fig. 1.
The main stages of the proposed cell tracking system.

Fig. 2.

(a) Association cases considered in our tracking system (i) migration, (ii) mitosis, (iii) disappearance, or apoptosis, or leaving field of view, and (iv) appearance, or cells entering the field of view. (b) Examples of cell events occurring in two consecutive sample frames of N2DL-Hela2 dataset.

Fig. 3.
Examples of clusters of cells detected as a single cell before pre-tracking.

Fig. 4.

Stage-wise example of cell cluster separation on frame #74 of N2DL-Hela02 dataset. The magnified parts of the image show the delineated cells before and after separation.

Fig. 5. CLGOF estimate indicated by green arrows showing cell mitosis (first and second row), and cell displacement (third row) between frames 8 (first column) and 9 (second column). The third column displays the difference between cell indicator functions for frames 8 and 9 of N2DH-SIM05 dataset.

Fig. 6. Estimation of vector field $\hat{\delta}$ displayed on current frame (left), next frame (middle) and warped frame using estimated $\hat{\delta}$ to be used for cell matching (right). Top row: $t = 10$ of N2DH-SIM05 dataset. Bottom row: $t = 41$ of N2DH-SIM04 dataset.

Fig. 7.

Example of possible cell association likelihood values between cells of warped frame at $t + 1$ and previous frame at t . The maximum likelihood matches are displayed in bold. The reject option yields the newly appearing cell that has no match with previous frame.

Fig. 8.
Comparisons between tracking performance values over all the tested image sequences.

Fig. 9.

Cell lineage trees automatically generated by our method on Fluo-N2DH-SIM06 dataset using manual cell segmentation and automated tracking (left) and fully automated cell segmentation and tracking (right).

Fig. 10.

Cell lineage tree generated by our fully automated method on the dense dataset Fluo-Hela01 dataset. This case yields $TRA=0.816$.

(a)

(b)

Fig. 11.
Cell tracks in 2D, 2D+t automatically generated by our method on Fluo-N2DH-SIM06 dataset.

Fig. 12. 2D and 2D+t displays of all cell trajectories of Fluo-C2DL-MSC02 in (a) and (c), MSD function versus time and the MSD mean over time for each cell of the C2DL-MSC02 dataset in (b) and (d).

TABLE 1

TRA values obtained from our automated tracking applied to reference segmentation masks.

Dataset name	Size	Number of Frames	TRA_{GT}
N2DH-SIM01	494x534	56	0.999
N2DH-SIM02	569x593	100	0.998
N2DH-SIM03	606x605	100	0.997
N2DH-SIM04	673x743	56	0.999
N2DH-SIM05	597x525	76	0.988
N2DH-SIM06	655x735	76	0.997
C2DL-MSC01	992x832	48	0.999
C2DL-MSC02	1200x782	48	0.973
N2DL-HeLa01	1100x700	92	0.992
N2DL-HeLa02	1100x700	92	0.970
N2DH-GOWT101	1024x1024	92	0.998
N2DH-GOWT102	1024x1024	92	0.996

TABLE 2

TRA and *DSC* values obtained from our automated joint segmentation and tracking method denoted by ST-Diff-TCV-Tr.

Dataset	<i>DSC</i> ($\mu \pm \sigma$)	<i>TRA</i>
N2DH-SIM01	0.926 ± 0.050	0.963
N2DH-SIM02	0.942 ± 0.012	0.949
N2DH-SIM03	0.939 ± 0.027	0.964
N2DH-SIM04	0.934 ± 0.013	0.964
N2DH-SIM05	0.941 ± 0.014	0.940
N2DH-SIM06	0.960 ± 0.026	0.973
C2DL-MSC01	0.762 ± 0.034	0.853
C2DL-MSC02	0.805 ± 0.043	0.584
N2DL-HeLa01	0.825 ± 0.042	0.816
N2DL-HeLa02	0.865 ± 0.005	0.845
N2DH-GOWT101	0.903 ± 0.012	0.913
N2DH-GOWT102	0.918 ± 0.010	0.914

TABLE 3

Comparisons between tracking performance values over all the tested image sequences.

Method	TRA				$\mu \pm \sigma$
	N2DH-SIM	C2DL-MSC	N2DL-HeLa	N2DH-GOWT1	
FR-Ro-GE	0.975	0.691	0.976	-	0.661 ± 0.399
HD-Har-GE	0.703	0.589	0.986	0.906	0.796 ± 0.206
IMCB-SG_1	0.907	0.633	0.915	0.882	0.834 ± 0.131
KTH-SE	0.957	0.763	0.991	0.976	0.922 ± 0.107
UP-PT	0.896	0.478	0.972	0.875	0.805 ± 0.208
CTC_ALL	0.526	0.277	0.595	0.516	0.478 ± 0.139
CTC_T3	0.920	0.625	0.959	0.911	0.854 ± 0.154
ST-Diff-TCV-Tr	0.959	0.719	0.831	0.914	0.856 ± 0.105