Extended-Hungarian-JPDA: Exact Single-Frame Stem Cell Tracking

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***Abstract*—The fields of bioinformatics and biotechnology rely on the collection, processing and analysis of huge numbers of biocellular images, including cell features such as cell size, shape, and motility. Thus, cell tracking is of crucial importance in the study of cell behaviour and in drug and disease research. Such a multitarget tracking is essentially an assignment problem, NP-hard, with the solution normally found in practice in a reduced hypothesis space. In this paper we introduce a novel approach to find the exact association solution over time for single-frame scan-back stem cell tracking. Our proposed method employs a class of linear programming optimization methods known as the Hungarian method to find the optimal joint probabilistic data association for nonlinear dynamics and non-Gaussian measurements. The proposed method, an optimal joint probabilistic data association approach, has been successfully applied to track hematopoietic stem cells.**

***Index Terms*—Cancer research, data association, Hungarian, linear programming, optimization, primal dual, segmentation, stem cell, tracking.**

I. Introduction

MULTITARGET tracking, the association of detected points into sequences over time, is an important NP-hard problem. Considerable efforts have been conducted to design tractable methods by reducing its complexity. These methods include nearest neighbor [1], joint probabilistic data association (JPDA) [2], [3] and multihypotheses data association [2], [4]. The common task among all tracking methods is to reduce the hypothesis space, the set of plausible association solutions, and to solve the association problem by selecting the most likely hypothesis, normally yielding a suboptimal solution. Solving the association problem in a reduced hypothesis space raises some important questions, such as the likelihood of finding the optimal solution in the reduced space, and the closeness of the optimal solution to the reduced space.

Multitarget tracking has a broad range of applications including some well-known applications such as air traffic control [5], robot control [6], [7], ocean surveillance [8], and some recent applications such as automated vehicle control [9] and, of particular interest to this paper, cell tracking [10], [11].

Recent advances in cell culturing and imaging allows the automated acquisition of huge sets of images, however methods of image analysis have not kept pace. Manual methods for analyzing such huge numbers of images to infer cell features such as size, shape, and motility are so onerous that automated methods of cell tracking and segmentation are in high demand. Applying advanced segmentation and tracking techniques to localize and track cells in long digital image sequences can improve our understanding of cellular behaviour. As a result, automated analysis methods would address fundamentally new questions in proteomics, genomics and stem-cell research [12]–[17].

A key development of the method proposed in this paper is to find an exact solution for the one scan-back tracking problem, applied to track living stem cells in multicellular phase contrast image sequences taken from blood stem cells [18]. The object of this particular project [18] is the analysis of stem-cell behaviour and differentiation, the process by which stem cells specialize to different cell types, a process which is crucial to understand if stem cells are to be used in cell and tissue regeneration. Specifically, given a culture of cells, observed over time, we need some way of determining whether a given cell is likely to die, to cause cancer, to specialize into an incorrect tissue type or, desirably, to specialize into the correct cell type, a determination which might be inferred from the statistics of many, many tracked cells.

II. Measurements

Our research interest in multitarget tracking stems from our goal toward developing a fully automatic cell tracking system. Two fundamental tasks are needed to accomplish this goal: the detection/recognition of cells in each frame, and the subsequent association of the detected cells over time.

The first task is essentially one of anomaly detection: the localization of groups of pixels inconsistent with the random behaviour of the image background. The blood stem cells in our study have a fairly regular shape and brightness pattern, thus in our previous work [19], [20] we exploited this useful information to design an effective cell localization/segmentation method to take an image I_k from a given sequence $I_{1:K} = \{I_1, I_2, \ldots, I_K\}$ and to infer measurements of M_k cell locations $Z_k = \{z_k^m | m \in [1, M_k]\}$. The measurement z_k^m, which encodes cell location (x_k^m, y_k^m) and radius r_k^m is discriminated by three criteria

$$P\left(I_k | z_k^m\right) = P_{\mathrm{cb}}\left(z_k^m\right) \cdot P_{\mathrm{in}}\left(z_k^m\right) \cdot P_{\mathrm{cdf}}\left(z_k^m\right) \qquad (1)$$

where P_{cb}, P_{in}, and P_{cdf} assess the presence of a cell in I_k at a size and location specified by z_k^m on the basis of testing the cell boundary, the cell interior, and the cell uniformity, respectively.

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III. Problem Statement

Aside from the cell radius, the blood stem cells which are of interest here all have the same visual appearance and cannot be discriminated visually. Therefore, to track a particular cell over time, the association task becomes crucial.

We denote by $F_{1:K}$ a possible hypothesis of the K-frame association problem

$$F_{1:K} = \{f_1, f_2, \ldots, f_K\} \tag{2}$$

where f_k is a parametric representation of frame k

$$f_k = \left\{ \left(l_k^j, u_k^j, s_k^j \right),\ 1 \le j \le J_k \right\} \tag{3}$$

where j indexes the J_k cells present in frame k, l_k^j is the label of the associated parent cell in frame $k-1$, $u_k^j = (x_k^j, y_k^j, r_k^j)$ specifies the cell radius r_k^j and location (x_k^j, y_k^j), and s_k^j is the cell age, updated as

$$s_k^j = \begin{cases} 1, & \text{if } \exists i \text{ such that } l_k^j = l_k^i \text{(i.e. cell split)} \\ \left(s_{(k-1)}^{l_k^j} \right) + 1, & \text{otherwise.} \end{cases} \tag{4}$$

The cell dynamics, affecting the relationship of (x_k^j, y_k^j) with $(x_{k-1}^{l_k^j}, y_{k-1}^{l_k^j})$ will, in general, be cell-type specific and may further be influenced by environmental factors, chemical gradients etc. In our context there are no deliberate experimental biases, and successive image frames are spaced so far apart in time (several minutes), therefore, inter-frame cell motion is independent over time, and a Gaussian random walk was found to well-approximate hand-tracked cell motion.

Note that because of the possibility of spurious or missed detections, it is not required that J_k equal M_k.

A. Solution of NP-Hard Problems

The optimum answer to the association problem is the maximum *a posteriori* estimation of $F_{1:K}$

$$\hat{F}_{1:K} = \arg \left\{ \max_{F_{1:K}} P(F_{1:K} | Z_{1:K}) \right\} \tag{5}$$

which is NP-hard, with a complexity exponential in K, so to find the optimal solution is essentially impossible. Instead, to solve the association problem, different methods have been introduced to solve the problem suboptimally either by finding the most likely hypothesis from a limited hypothesis set over multiple frames

$$\left\{ F_{1:K}^h | h = 1, 2, \ldots, \right\} \tag{6}$$

thus finding the best member

$$\hat{F}_{1:K} = F_{1:K}^{\hat{h}} \text{ where } \hat{h} = \arg \left\{ \max_h P\left(F_{1:K}^h | Z_{1:K} \right) \right\} \tag{7}$$

as the solution such as the multihypothesis tracking (MHT) algorithm [4], or a frame by frame solution based on single-frame associations over time $[k-1, k]$ such as JPDA [2].

The single-frame association method is a feasible approach which has been widely used. However even in the single-frame case, virtually all approaches propose to find solutions over a reduced hypothesis space for frame k

$$\left\{ f_k^h | h = 1, 2, \ldots, \right\} \tag{8}$$

and searching for the best member

$$\hat{f}_k = f_k^{\hat{h}} \text{ where } \hat{h} = \arg \left\{ \max_h P\left(f_k^h | Z_{1:k} \right) \right\} \tag{9}$$

as the solution. The optimal single-frame solution is found if it is included among the hypotheses of that frame, i.e., if

$$\arg \left\{ \max_{f_k} P(f_k | Z_k) \right\} \in \left\{ f_k^h \right\}. \tag{10}$$

The key, here, to efficiency is to minimize the number of hypotheses; the key to the quality of estimation to include many likely hypotheses in the hypothesis set. As these goals are in opposition, we are left with a complexity/quality trade off. One of the widely used and well known single-frame algorithms to solve the multitarget tracking problem in a reduced hypothesis space is JPDA [2], discussed next.

B. Joint Probabilistic Data Association

To solve (5), JPDA has been widely applied for multitarget tracking [2], [3], [21], [22]. Assuming linear dynamics and Gaussian measurements, standard JPDA employs a Kalman filter to model the association uncertainties [1]–[3], [22]. There have been considerable efforts to generalize JPDA and overcome its shortcomings by using the extended Kalman filter (EKF) to linearize modest nonlinear systems [21], [23]–[26]. JPDA assumes the number of targets T to be known with the following constraints: 1) each measurement originates from only target or clutter; 2) a measurement can be associated at most to one target; and 3) at most one measurement can be associated to a target.

To make the association problem tractable, JPDA reduces the number of possible association hypotheses and keeps a reasonable subset of them as valid association hypotheses using a gating strategy which JPDA employs to validate the measurements and to generate a subset of association hypotheses, valid associations, based on validated measurements. Gating keeps the measurements which fall inside the validation gate of each target as valid measurements, hence the measurements that fall outside of the target's validation gate are not considered as association candidates and are thrown away.

Moreover to manage the dimensionality and so the complexity of the problem, JPDA employs a recursive strategy and updates the filter distribution for each target, i.e., JPDA sequentially estimates the marginal distribution $P(f_k | Z_{1:k})$ given the measurements $Z_{1:k}$. From Bayes' rule

$$P(f_k | Z_{1:k}) = \frac{P(f_k | Z_{1:k-1}) \cdot P(Z_k | f_k, Z_{1:k-1})}{P(Z_k | Z_{1:k-1})}. \tag{11}$$

The information of the measurements $Z_{1:k-1}$ has already been used to generate hypotheses $f_{1:k-1}$, thus all of the information of $Z_{1:k-1}$ is implicitly embedded in f_k so that

$P(Z_k|f_k, Z_{1:k-1})$ can be simplified to $P(Z_k|f_k)$. Moreover $P(Z_k|Z_{1:k-1})$ is a fixed constant so we can write

$$P(f_k|Z_{1:k}) = \lambda_k \cdot P(f_k|Z_{1:k-1}) \cdot P(Z_k|f_k) \qquad (12)$$

where λ_k is a normalization constant. The first term of (12), $P(f_k|Z_{1:k-1})$, is a prediction step. Because of the linear dynamics and Gaussian measurement assumption, the Kalman filter is considered for the prediction step in standard JPDA.

The second term of (12), $P(Z_k|f_{k,j})$, is the likelihood of measurement Z_k given hypothesis $f_{k,j}$ and is computed in standard JPDA as

$$P(Z_k|f_{k,j}) = \left[\beta_j^0 + \sum_{m\in[1,M_k]} \beta_j^m \cdot P\left(z_k^m|f_{k,j}\right)\right] \qquad (13)$$

where β_j^m is the marginal posterior probability of associating measurement m to target j so that β_j^0 is the probability of no measurement to be associated to target j.

IV. Proposed Method

As was mentioned in the previous section, JPDA has been extensively used as a single-frame solution for multitarget tracking problem. Most of the single-frame tracking methods, including JPDA, reduce the number of possible association hypotheses to make the association problem tractable. The major shortcoming of these methods is finding the suboptimal single-frame solution over a reduced hypothesis space. The other disadvantages of JPDA are the assumptions of linear dynamics and Gaussian measurements.

An optimal general-purpose single-frame approach must solve the association problem over the entire single-frame hypothesis space and assume no restrictions on dynamics and measurements. To achieve this goal and to derive an algorithm to be applicable to the stem-cell problem at hand in which dynamics and measurements are, respectively, assumed nonlinear and non-Gaussian, a nonlinear, non-Gaussian method is proposed to solve the tracking problem. A feasible optimal method is proposed to evaluate all possible hypotheses, by representing the tracking problem in the form of an assignment problem and then by extending a linear programming optimization method known as the Hungarian method [27] to apply to the problem at hand.

Network programming and optimization, as a branch of operations research, has seen considerable research attraction. A special case of this class of problems is the assignment problem, which has been solved using a class of linear programming methods known as primal-dual algorithms [28], of which the Hungarian method is an example. An assignment problem of order n is the one-to-one connection or assignment of n sources to n sinks. The solution can be represented by a binary matrix of order $n \times n$, as illustrated in Fig. 1 for $n = 3$.

In this section, we will represent the tracking problem in the form of an assignment matrix by considering the cells (previous frame) and measurements (current frame) as the row and column indexes, respectively. First the proposed nonlinear, non-Gaussian method follows, then we discuss how the proposed formula can be represented and solved as a generalized assignment problem.

		Sinks. A	B	C
	1	1	0	0
Sources	2	0	0	1
	3	0	1	0

Fig. 1. Example assignment matrix: three sources 1, 2, 3 need to be assigned to three sinks A, B, C. Clearly each row and column of the matrix must sum to one.

A. Nonlinear Non-Gaussian JPDA (NNJPDA)

In standard JPDA new tracks can not be initiated, the number of targets is known, dynamics are linear, measurements are Gaussian and the Kalman filter is used to predict the new state. In contrast, in blood stem cell tracking new tracks for divided cells must be initiated, the number of cells is unknown, the dynamics are a constrained random walk (nonlinear due to motion constraints imposed by nearby cells), and due to clutter the measurements are non-Gaussian. The nonlinear, non-Gaussian nature of problem makes the Kalman filter an inappropriate choice to predict the new state.

The basic measurement constraints are as follows: 1) each measurement originates from only target (cell) or clutter; 2) each measurement can be associated to one cell; and 3) up to two measurements in frame k can be associated to the same cell in frame $k-1$, where this last constraint differs from standard JPDA.

Each cell in the state f_k must belong to one of the following sets:

$$\begin{aligned} &\text{Unassociated :} && U = \left\{j|l_k^j = 0\right\} \\ &\text{Split :} && S = \left\{j|l_k^j = l_k^i \text{ for } j \neq i\right\} \\ &\text{Regular :} && R = \{j|j \notin \{U \cup S\}\}. \end{aligned}$$

To evaluate the association solution $\hat{f}_k$, a measurement set Z_k is obtained to update the previous state estimate $\hat{f}_{k-1}$ by selecting the hypothesis with the maximum joint association probability among all possible hypotheses for that frame. From the derivation of $P(f_k|Z_{1:k})$ in (12), conditioning on both sides we have

$$\begin{aligned} P(f_k|Z_{1:k}, f_{k-1}) &= \lambda_k \cdot P(f_k|Z_{1:k-1}, f_{k-1}) \cdot P(Z_k|f_k, f_{k-1}) \\ &= \lambda_k \cdot P(f_k|f_{k-1}) \cdot P(Z_k|f_k) \end{aligned} \qquad (14)$$

where the first term explicitly examines the likelihood of f_k given f_{k-1}, which involves a dependence on cell dynamics, splitting, and separation probabilities:

$$P(f_k|f_{k-1}) = \lambda_a \cdot (\text{Dynamics}) \cdot (\text{Splitting}) \cdot (\text{Separation}). \qquad (15)$$

The second term of (14) assesses the likelihood of the measurement Z_k given the cell state f_k. Because, in our proposed method, any hypothesis for f is directly derived from Z, every measured point, whether correctly or falsely detected, corresponds to a cell in hypothesis f_k. Therefore, the computation of $P(Z_k|f_k)$ simplifies to counting the number of cells in f_k

which are associated (thus correctly detected) or un-associated (thus a false alarm)

$$P(Z_k|f_k) = \lambda_b \cdot (\text{Detect}) \cdot (\text{False Alarm}) \tag{16}$$

with the details of (15) and (16) in place, (14) becomes

$$\begin{aligned} P(f_k|Z_{1:k}, f_{k-1}) = \lambda_k \cdot & \left[\prod_{j\in R\cup S} P_{\text{Dyn}}\left(f_{k,j}|f_{k-1,l_k^j}\right)\right] \\ & \cdot \left[\prod_{j\in S} P_{\text{Split}}\left(s_{k-1,l_k^j}\right)\right] \\ & \cdot \left[\prod_{j\in R\cup S} P_{\text{Sep}}\left(\{f_{k,j}\}, j \in R\cup S\right)\right] \\ & \cdot \left[\prod_{j\in R\cup S} P_{\text{Detect}}\right] \cdot \left[\prod_{j\in U} P_{\text{False}}\right] \end{aligned} \tag{17}$$

where j indexes the cells in one of the R, S, and U sets containing mature cells, divided cells and false alarms, respectively. P_{Detect} is the probability of cell detection which is a constant, set to 0.9 empirically based on the performance of the cell model [19], [20]. P_{Dyn} represents the cell motion dynamics to assess a hypothetical cell j in frame k based on its location at time $k-1$. Based on hand-tracked cell motion, a constrained Gaussian random walk well approximates the observed cell dynamics. Because there is no explicit prediction of the statistics from $k-1$ to k, rather an assessment of a given set of hypotheses at frame k relative to $k-1$, therefore, nonlinear dynamics can readily be accommodated.

Stem cells can split and bear new cells. A typical stem cell must be mature before it can split, i.e., there is an age constraint based on which the likelihood of cell division can be estimated. Cell divisions associated to young stem cells, below some minimum age, are considered unlikely and will be penalized by P_{Split}. After the cell's age passes the minimum age constraint, the probability of cell-split will increase with increasing cell age. Thus, P_{Split} predicts the cell division in frame k based on the cell age in frame $k-1$.

P_{False} is a penalty for the association of false alarms: Any hypothesis containing extra measurements which are not associated to a cell is unlikely and so should be penalized accordingly. Finally stem cells in our experiments can not overlap, hence the centres of two nearby cells cannot be closer than the sum of their radii. P_{Sep} is the cell-distance penalty based on the cell center separation, and sets to zero the likelihood of any hypothesis containing inadmissibly close cell centers. As was discussed after (15), because any hypothesis for f is directly derived from Z, and because the inference of Z [19], [20] does not allow the creation of measurements closer than a specified minimum separation, therefore, the P_{Sep} term is, in practice, only a formality.

Having the association problem specified by (17), the optimization problem is to find the best estimate among all possible hypotheses evaluated. We propose to use an extended version of the Hungarian method (E-Hungarian), discussed next.

B. The Hungarian Method

The Hungarian method as a primal-dual algorithm belongs to the class of linear programming methods which have been used for the assignment problem [28]. The basis of the Hungarian method was introduced by Egervary and Konig and it has been completed later by Kuhn [27]. Primal-dual algorithms are characterized by

- A primal vector and a dual feasible solution is maintained by the algorithm.
- One of the following tasks is performed by the algorithm in each iteration.
  1) The primal vector is kept fixed and the dual feasible solution is changed.
  2) The dual solution is kept fixed and the primal vector is changed toward primal feasibility while satisfying the present dual solution.
- By iterating the algorithm, the primal vector progresses toward primal feasibility.

We wish to solve the tracking problem represented by matrix $\mathtt{F} = (\mathtt{F}_{jm})$ given the cost matrix $d = (d_{jm})$. Each element of the cost matrix represents the cost of associating measurement m to cell j. To solve the assignment problem we need to minimize

$$A(\mathtt{F}) = \sum_{j=1}^{J_k}\sum_{m=1}^{M_k} d_{jm}\mathtt{F}_{jm} \tag{18}$$

subject to

1) $\sum_{m=1}^{M_k} \mathtt{F}_{jm} = 1\ \forall j \in [1, J_k]$. Each row of F sums to one.
2) $\sum_{j=1}^{J_k} \mathtt{F}_{jm} = 1\ \forall m \in [1, M_k]$. Each column of F sums to one.
3) $\mathtt{F}_{jm} \geq= 0\ \forall j \in [1, J_k]\ \&\ m \in [1, M_k]$ F is nonnegative.
4) $\mathtt{F}_{jm} = 0\ or\ 1\ \forall j \in [1, J_k]\ \&\ m \in [1, M_k]$. Each element of F is 0 or 1.

Each feasible solution of (18) is an assignment problem of order $J_k \times M_k$. The dual of (18) is to find $\delta = (\delta_1, \delta_2, \ldots, \delta_{J_k})$ and $g = (g_1, g_2, \ldots, g_{M_k})$ such that

$$B(\delta, g) = \sum_{j=1}^{J_k} \delta_j + \sum_{m=1}^{M_k} g_m \tag{19}$$

is maximized, subject to

$$\delta_j + g_m \leq d_{jm},\ \ j \in [1, J_k]\ \&\ m \in [1, M_k]. \tag{20}$$

The constraint in (20) can be rewritten as

$$\bar{d}_{jm} = d_{jm} - \delta_j - g_m \geq 0,\ \ j \in [1, J_k]\ \&\ m \in [1, M_k] \tag{21}$$

which is called the dual feasibility condition for (δ, g), where $\bar{d} = (\bar{d}_{jm})$ is the reduced cost matrix and its elements $\bar{d}_{jm}$ are the reduced cost coefficients. As a result the vectors (δ, g) are dual feasible if and only if the reduced cost matrix $\bar{d} \geq 0$ [28]. An assignment problem F and a dual feasible solution (δ, g) are optimal if

$$\mathtt{F}_{jm}(d_{jm} - \delta_j - g_m) = \mathtt{F}_{jm}\bar{d}_{jm} = 0,\ \ j \in [1, J_k]\ \&\ m \in [1, M_k] \tag{22}$$

which is called the complementary slackness optimality condition for the assignment problem and its dual. These are the basic assumptions in the Hungarian method such that it begins with a dual feasible solution and tries to find an assignment with allocations among the cost matrix elements which satisfy

$$\bar{d}_{jm} = d_{jm} - \delta_j - g_m = 0. \tag{23}$$

These elements of d are known as admissible elements. To update the cost matrix after each dual solution change in each stage, reduced cost coefficients are computed once, thus computational complexity per stage is at most $\bigcirc(n^2)$. As a result the overall computational complexity of the Hungarian method with consideration of n stages will be $\bigcirc(n^3)$ [28].

C. Extended Hungarian JPDA

The tracking problem can be represented in the form of an assignment problem so that a primal-dual algorithm can be applied to solve it. To represent the tracking problem in the form of an assignment matrix, the measurements Z_k of frame k (hypothesized cell locations) are assigned to the estimated cells $\hat{f}_{k-1}$ of frame $k-1$, giving rise to an assignment matrix (see Fig. 1) which represents the association of measurement $m \in [1, M]$ to cell $j \in [1, J]$.

Although the Hungarian method efficiently and optimally solves single-frame assignment problems, it is insufficiently general to solve the cell tracking problem of interest because the one-to-one assignment precludes cell division and false alarms. In particular, recall the simple example of Fig. 1 in which the measurements A, B and C in the current frame are assigned, respectively, to the cells 1, 3, and 2 in the previous frame. To allow one of the cells (1, 2, 3) to split or one of the measurements (A, B, C) to be false requires a different approach. The key concept of this paper is that cell splits and false alarms can still be accommodated in a one-to-one assignment problem by enlarging the assignment matrix.

In our proposed E-Hungarian method, we double the number of rows to allow cell splitting and then add extra rows with the same number of measurements to allow false alarms. To allow mis-detection and to make the tracking matrix square, new columns are added. In this way the copied rows ($\bar{1}$, $\bar{2}$ and $\bar{3}$ in the example in Fig. 2) allow cell splitting so that by duplicating each row up to two measurements in the current frame can be associated to the same cell in the previous frame (represented by the rows) while each measurement assigned to the extra rows (dummy cells) will be interpreted as false alarms. Similarly the extra columns represent dummy measurements; any assignment from cell rows to these columns will be considered mis-detection, implying that one of the cells in frame $k-1$ is not associated to frame k.

To solve the cell association problem by the proposed method, we embed the proposed NNJPDA (17) as cost function d in the E-Hungarian method, so that each element of which (d_{jm}) represents the cost of assigning the measurement m in time k to the target j from time $k-1$. The goal is minimizing the cost of joint association of targets to measurements. To derive the cost matrix d from (17) we compute the following.

		Meas.			Dummy Meas.					
		A	B	C	D	D	D	D	D	D
Cells	1	0	0	0	0	0	0	0	1	0
	2	0	0	1	0	0	0	0	0	0
	3	0	0	0	1	0	0	0	0	0
Cell Copies	$\bar{1}$	0	0	0	0	0	1	0	0	0
	$\bar{2}$	1	0	0	0	0	0	0	0	0
	$\bar{3}$	0	0	0	0	1	0	0	0	0
Dummy Cells	D_c	0	0	0	0	0	0	0	0	1
	D_c	0	1	0	0	0	0	0	0	0
	D_c	0	0	0	0	0	0	1	0	0

Fig. 2. Tracking matrix illustrating splitting and unassociation. In this example, measurements A and C are both assigned to cell 2 (showed by rows 2 and $\bar{2}$), measurement B is assigned to a dummy cell. The conclusions are that cell 2 has split, cells 1 and 3 are undetected, and measurement B is a false alarm.

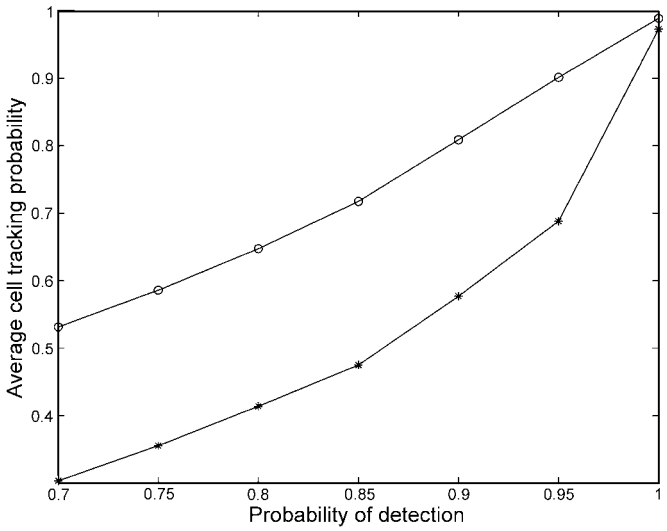

Fig. 3. Tracking performance. E-Hungarian-JPDA(o) versus NN($*$) as a function of the probability of detection (P_{Detect}). For each value of probability of detection, 200 video clips each of 50 frames have been used.

1) $\text{Dyn}_{jm} = [1/(P_{\text{Dyn}}(z_k^m | u_{k-1}^j))]$
2) $\text{Split}_{jm} = [1/(P_{Split}(s_{k-1}^j)^2)]$
3) $\text{Detect} = [1/P_{\text{Detect}}]$
4) $\text{False} = [1/P_{\text{False}}]$

Then each element d_{jm} of the cost function d is obtained by

$$d_{jm} = \begin{cases} \text{Dyn}_{jm} \times \text{Detect} \\ \quad \times \text{False} & j \in \{\text{Cells}\} \\ \text{Dyn}_{jm} \times \text{Split}_{jm} \\ \quad \times Detect \times \text{False} & j \in \{\text{Cell Copies}\} \\ P_{\text{Unassociation}} & j \in \{\text{Dummy Cells}\} \\ P_{\text{Unassociation}} & m \in \{\text{Dummy Meas.}\} \end{cases} \tag{24}$$

where we note that the P_{Sep} term of (17) does not appear, having been satisfied inherently by the measuring process. Finally having d as the cost function, the proposed E-Hungarian-JPDA finds the optimal assignment by satisfying (22).

By minimizing the cost function d we are maximizing the NNJPDA in (17). Therefore, the optimal association among all possible hypotheses is found by employing E-Hungarian method to solve (17) and the exact solution for frame k given $(k-1)$ is obtained.

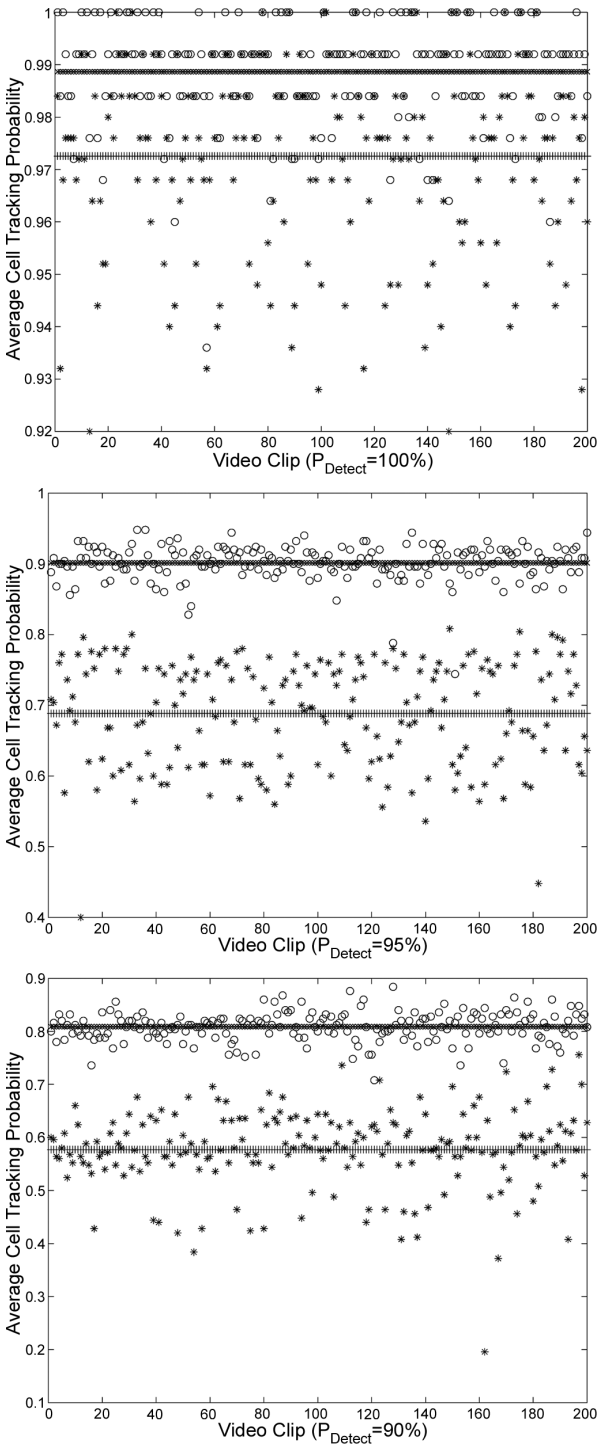

Fig. 4. Tracking performance of E-Hungarian-JPDA(o) versus NN($*$). Top: perfect detection ($P_{\mathrm{Detect}} = 100\%$). Middle: ($P_{\mathrm{Detect}} = 95\%$). Bottom: ($P_{\mathrm{Detect}} = 90\%$). The average probabilities over 200 video clips are superimposed.

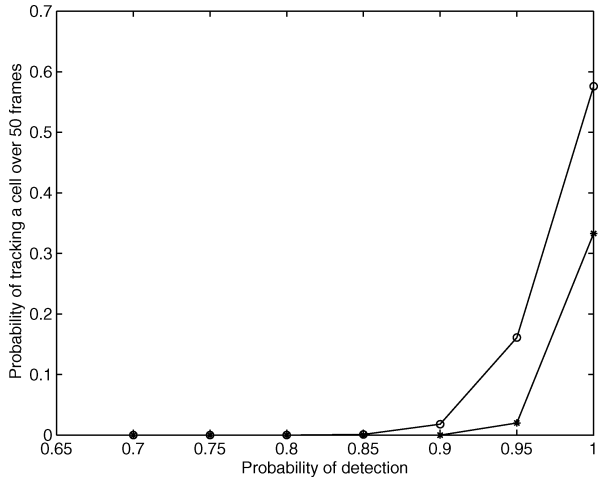

Fig. 5. Probability of perfect tracking in which a typical cell is tracked over all frames from the first to the last frame. E-Hungarian-JPDA(o) versus NN($*$) as a function of the probability of detection (P_{Detect}). In each case 200 video clips each of 50 frames are used.

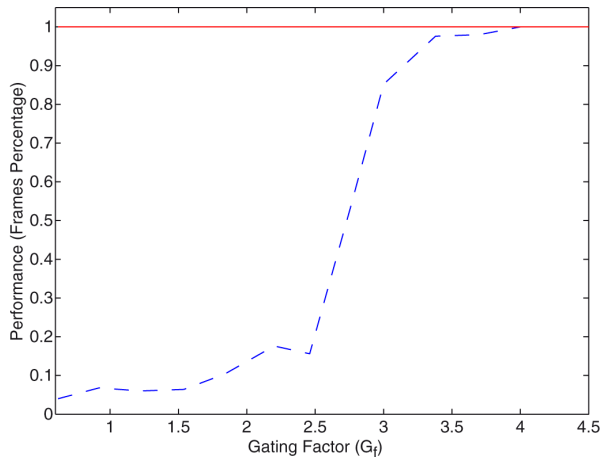

Fig. 6. Performance of the proposed E-Hungarian-JPDA (solid line) in comparison with standard JPDA (dashed line) as a function of gating factor (G_f).

V. Results

We have measured the performance of the proposed method and compared it with nearest neighbour (NN) [1] and standard JPDA [2]. Because of the difficulty in obtaining association ground truth for laboratory videos and because we wish to distinguish between errors in cell detection (addressed in [19] and [20], and not the focus of this paper) and cell association, therefore, synthetic video clips simulating the random behaviour of stem cells were generated, in which nonoverlapping cell dynamics were applied. We generated 1400 video clips, each video clip composed of 50 frames and 5 cells. The cells do not split to allow methods such as NN and standard JPDA, which do not support splitting, to be tested. We propose to assess the performance of the algorithm based on the average percentage of frames in which a cell has been correctly associated in comparison with ground truth.

The performance of the proposed method in comparison with NN is depicted in Fig. 3. The two methods are compared for different values of the probability of detection (P_{Detect}). For each value of P_{Detect}, 200 video clips, each composed of 50 frames, are generated and the synthetic cell centres are tracked over time applying the proposed method and NN. As we can observe, the proposed method has outperformed NN for all values of P_{Detect}. A comparison of the two algorithms for $P_{\mathrm{Detect}} = 100\%$, $P_{\mathrm{Detect}} = 95\%$ and $P_{\mathrm{Detect}} = 90\%$ is depicted in

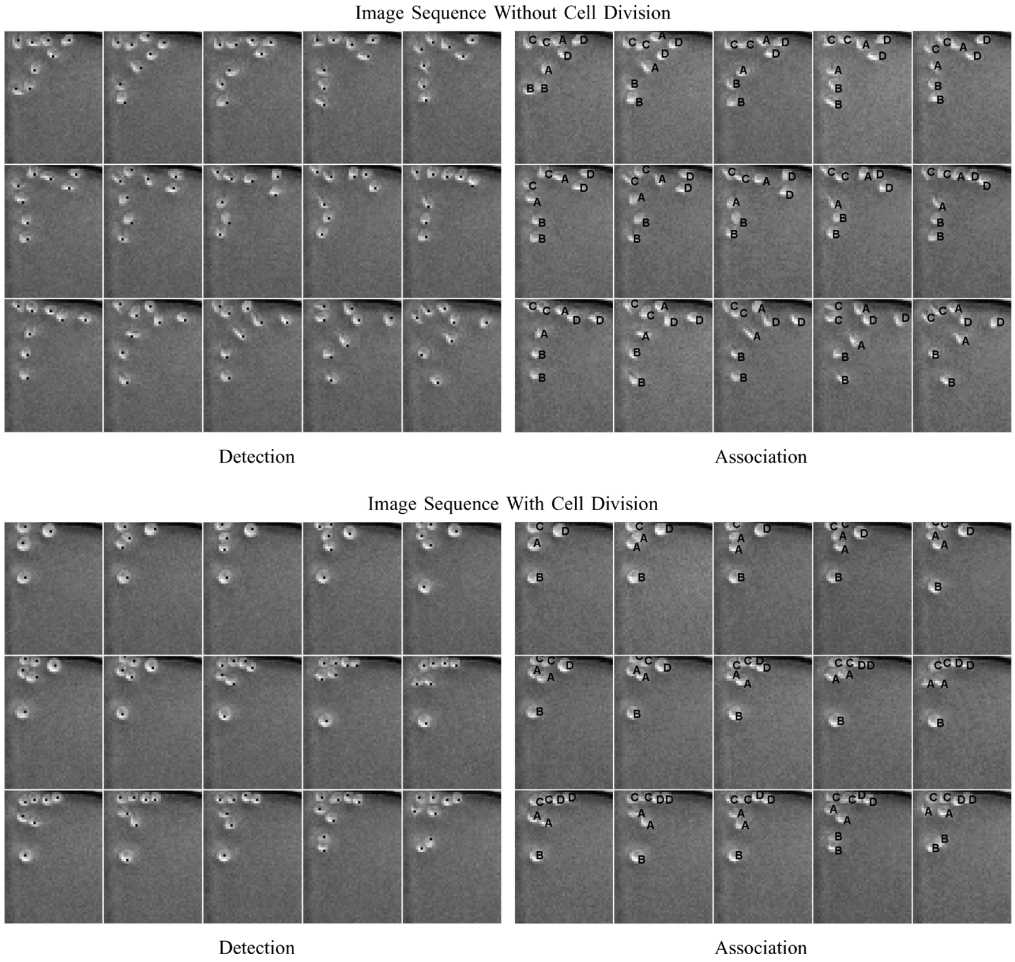

Fig. 7. Example illustrating the detection and association by applying the proposed cell model in [19] and [20] and the proposed E-Hungarian-JPDA, respectively. The detection results are superimposed on the original HSCs as black dots locating the detected cell centres. The association results are also superimposed on the original HSCs such that each letter shows a different cell track/split over time.

greater detail, sequence by sequence, in Fig. 4—top, middle, and bottom, respectively.

Fig. 5 plots the probability of perfect tracking of a cell over all 50 frames. The average tracking probability is computed over 200 video clips for each value of P_{Detect} (overall 1400 video clips composed of 50 frames). As can be seen the proposed method performs much better than NN, especially for $P_{\text{Detect}} > 90\%$.

Fig. 6 shows the performance of the proposed method in comparison with the standard JPDA for $P_{\text{Detect}} = 100\%$. To compare the results, the performance of JPDA is measured as a function of gate area $G_v = \pi \times G_r^2$, where $G_r = G_f \times \sigma_{rw}$ is the gate radius, set to be a multiple (G_f) of the standard deviation of the constrained Gaussian random walk. Fig. 6 clearly shows that increasing G_f improves the performance of JPDA towards that of the E-Hungarian-JPDA, which is expected since for a sufficiently large G_f JPDA is testing all hypotheses. For $G_f = 4$, where the performance of the two methods is almost equal, the gating area is a circle with diameter of $2 \cdot G_r = 20$ pixels. Considering that each well is about 70×50 pixels, such a circular gate covers a significant fraction of the well's area around each cell centre. As the circular gate completely covers the field of measurements, standard JPDA evaluates all possible $n!$ hypotheses to find the best association to assign n sources to n sinks, much more expensive than E-Hungarian-JPDA.

To generate results using real data, we have applied the proposed method to long streams of microscopic phase contrast HSC video. First, the cell center candidates are located by applying the probabilistic cell model in [19] and [20] in which the cell candidates are found by locating and thresholding the local maxima in a cell probability map. Then, to track the cells over time, our proposed E-Hungarian-JPDA method has been applied to the localized cell centers as potential HSC candidates.

Fig. 7(top left) shows the detected nondividing cell centers in 15 frames of a HSC video clip spanning 45 min of time (successive frames 3 min apart). The proposed E-Hungarian-JPDA method is applied to the detected cell centres, with the tracking results depicted in Fig. 7(top right). As can be observed in Fig. 7, the proposed E-Hungarian-JPDA method is able to associate the nondividing HSCs correctly.

The proposed method is also capable of tracking more challenging *dividing* HSCs, which is not the case for standard JPDA. Fig. 7(bottom-left) again shows the detected dividing cell cen-

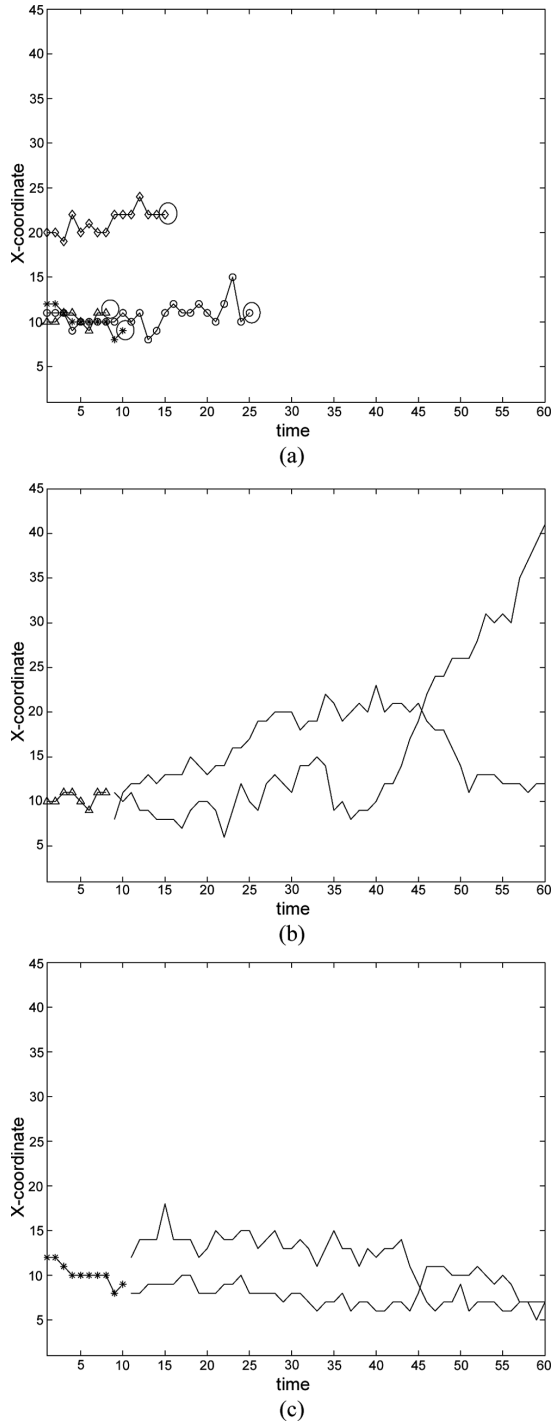

Fig. 8. Blood stem cell tracks over time. (a) Tracks of four mature cells from Fig. 7(bottom); the large circle at the end of each track highlights the division point over time when a mature cell divides to produce two new cells. (b) The triangled line represents the track of the mature cell from (a) before splitting, then dividing into two new tracks (solid lines). (c) As in (b), but for the starred-cell from (a), beginning as a mature cell and dividing into two.

ters in 15 frames of HSC video. The association results obtained by applying the proposed tracking method are depicted in Fig. 7(bottom-right). Again the dividing HSCs are tracked correctly by the E-Hungarian-JPDA method.

To see the tracking of splitting cells in greater detail, Fig. 8 shows the details of two dividing stem cells over time.

VI. Conclusion and Discussion

As an important application of multitarget tracking in biomedical research, this paper presents an optimal single-frame assignment solution for HSC tracking to associate HSCs in phase contrast microscopic images. Our proposed approach uses linear programming optimization, based on an extended Hungarian method. This is a generative algorithm and can be used with various tracking methods, including nearest-neighbor, PDA, JPDA, particle filtering, MHT, and deformable models, by designing the correct cost function.

The contribution of the paper is a generalization of the Hungarian method to allow association with track divisions, false alarms, and missed detections. We are motivated to consider alternative generalizations for other classes of tracking problems.

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