Review of Methods for Cell Tracking

Shreyasi Ghosh
Master Student
Fachbereich Informatik
Rheinland-Pfälzische Technische Universität Kaiserslautern
Email: ghoshs@rhrk.uni-kl.de

***Abstract*—Object tracking has many applications in various fields, especially in the biomedical domain. Cell tracking can be used to detect changes in patterns of cell movements which can then be used for accurate diagnosis of diseases and help in the development of medicines. There are different approaches available for cell tracking using traditional computer vision approaches and deep learning-based approaches. This survey focuses on different methods of cell tracking – detection by boundary box detection, tracking by segmentation, tracking by model evaluation, tracking by filtering, and point-based approach and the different datasets available for cell tracking.**

I. Introduction

Every living organism, including humans, develop from a single cell. The fertilized ovum undergoes multiple cycles of growth, division, migration, and differentiation to form a multicellular organism. Cell migration plays a major role in embryogenic development. Cell migration further enables wound healing by developing inflammatory responses via recruiting immune cells and maintaining tissue homeostasis like continuous replacement of intestinal epithelial cells, as well as red blood cells. It plays a significant role in developing various pathologies; for instance, faulty migration of neural crest cells leads to congenital syndromes like cleft lip/palate; excess migration (metastasis) of tumor cells can lead to spread of cancer in distant areas of the body; even various pathogens, including bacteria and viruses, can infect our body cells via cell migration [63]. Researchers can track cells and study their migration with the recent advancement of technologies. Cell tracking has become an essential tool in medical science for carrying out several purposes, for instance, studying embryogenic development, detecting pathophysiology of a disease, early and accurate diagnosis of a disease, as well as evaluating the extent of cancer metastasis [8].

An object can be differentiated from its surrounding background only if there is a contrast in light reflection between the object and its surroundings. Cells are usually transparent or translucent and do not display significant contrast with their surrounding media. Therefore, to detect cells, traditionally reflective light microscopes were used. In these microscopies, cells are fixed using a fixative and stained with a dye, for example, Hematoxylin and Eosin (HE) staining. The concept of staining is that the dye can only work if it has penetrated the cell membrane, which is possible by killing the cells with the help of fixatives. This enables dyed cells to reflect light, enabling researchers to detect cells. However, it is impossible to track cell migration with dead cells. Even electron microscopes visualize dead cells via fixation. However, with the discovery of transmitted light microscopy, fluorescence staining, fluorescence microscopy, and time-lapse imaging facility, researchers can visualize live cells and track their migration [67].

Object tracking, which includes cell tracking, finds its application in several spheres of biological research. Object tracking can be used in:

- Intracellular dynamic tracking – To track intracellular organelles and vesicles for analyzing cellular dynamics [73,74].
- Protein Tracking – To understand the place and dynamic of protein pairs interacting or proteins position [75,76].
- Tracking normal and malignant cell cycle – To track the behavior of cells and to find cells that are defective in their life cycle [77,78].
- Tracking of cell death – To study the tumor growth and to treat the response of malignancies [79].
- Cell behavior tracking – This includes tracking cellular behaviors that cause morphogenesis, such as division, migration, or death [80]-[82].

Analyzing cell images manually presents several difficulties, such as the large size of a single cell image sequence and diagnosing a disease depends on a long time, laborious work, and the experience of the operator.

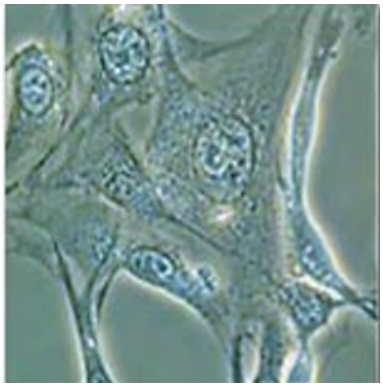
Although many efforts have been made to improve tracking, the small size of cells and their organelles raises numerous difficult issues appropriate for computational object-tracking systems [47].

II. Methods

Object tracking is locating objects and monitoring their behavior over time [8]. In cell tracking approaches, the image sequence of cells is acquired using time-lapse techniques at specific time intervals. Cell tracking aims to portray cell functions like migration, and engraftment at the organs, tissue, and molecular levels. It also provides insights into the biological processes of emerging cell-based therapies [10].

A lot of methods are being used widely for cell tracking:

1) **Tracking by detection (detection-based approach)** – In this approach, cells are detected in every frame of



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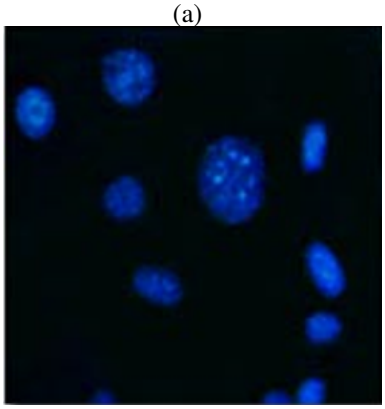

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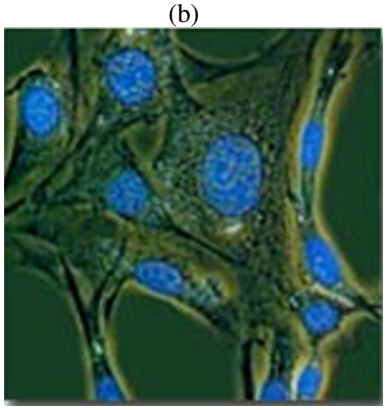


(c)

Fig. 1: Comparison between phase contrast and fluorescence microscope imaging [57].
(a) This figure depicts monolayer of fibroblast cell culture, imaged by phase contrast microscope; (b) This figure represents the same viewfield as in a, this time, the image has been taken using fluorescence microscope after staining the cells with DAPI stain; (c) This figure represents a combination of both phase contrast and fluorescent imaging of the same viewfield

the video independently based on intensity, texture, or gradient features [23]. Objects are matched between consecutive frames by objective functions. A bounding box is incorporated into trackers to train a detector, which detects bounding boxes, and an appearance model that trains the similarity of the bounding boxes [26]-[31]. LSTM (Long Short-Term Memory) [32] is used to measure the similarity among the time series of detected bounding boxes [33]-[37]. This type of approach does not use the spatial context outside of the bounding box [28].

The object detection stage is also achieved by the process of image segmentation. Segmentation is the assignment of different labels to every pixel in each image so that the pixels with the same label have particular particles. The segmentation type considered for cellular images are – semantic segmentation and instance segmentation [9]. For each known object in an image, semantic segmentation finds the cell category of each pixel. Instance segmentation identifies the cell with certain features for each pixel of every recognized object within an image.

Traditional approaches of image segmentation are categorized into four approaches – Thresholding, Region Growing, Edge Detection, and Pattern Matching [38].

- Thresholding – This is one of the most prevalent techniques for image segmentation. Thresholding produces a binary image as output where one condition will present the foreground objects, and the complementary condition will match the background [40]. Thresholding is effective and applicable to images having high contrast and uniform backgrounds, and the objects are homogenous. The Thresholding technique is complicated by a number of factors, including transient and mutual noise, diffuse brightness, the accumulating gray levels within the item and its background, insufficient contrast, and the object's size that is out of scale with the scene [8]. In reality, Thresholding alone produces insufficient segmentation results; hence, thresholding is used as a basic level in the pipeline in most cell segmentation techniques.
- Edge detection – Edge detection is the finding of an edge belonging to an extreme change in image intensity [43]. In comparison to thresholding, techniques of edge detection are stronger, but they miscarry on low levels of contrast image [42]. One of the drawbacks of both edge detection and thresholding is that they cannot recognize two objects of the same boundary and intensity. This drawback can be avoided by the watershed technique, where the image is considered a topographic relief, and the pixel gray levels are their altitudes [8]. Methods based on watershed segmentation have higher performance in differentiating clustered objects, but

their utility decreases in low contrast conditions, especially when local invisibility of the cell membrane occurs [41]. Specific pre and post-processing methods are normally used as this strategy can cause over-segmentation.

- Region growing – The region growing techniques depend on the connectivity, subregion features, and structural characteristics of objects [41]. This technique is divided into two steps- firstly, the selection of sub-regions having a uniform or near uniform properties, and secondly, the grouping of neighboring pixels to specify whether the pixels should be merged into the larger sub-region by a cost-minimizing function [24]. The procedures for a growing region that allow for both region splitting and region growing can be improved by merging and splitting tactics [41].
  The advantages of detection-based approaches are- working on lower imaging frequencies and advanced data association techniques used for tracking and separating the segmentation tasks [24]. The mutual independence of detection and association steps enables tracking new cells entering the field of view and forward-backward spatio-temporal data association [25].
  This method also has two drawbacks. Firstly, the performance of detection and association is independent which means objects are detected first, and then the detected object is associated between successive frames using one-to-one matching. This means the errors available in the detection steps proceed to the association steps. Secondly, they cannot extract long-term spatial and temporal context from multiple frames [38].

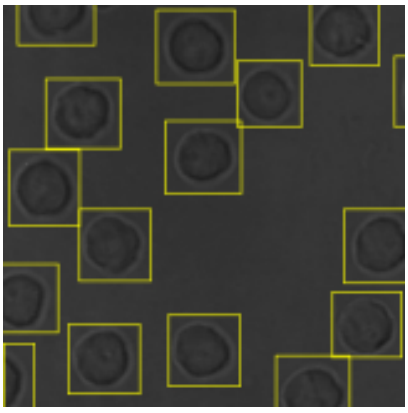


Fig. 2: Cell Detection via bounding box [9].

2) **Tracking by Model Evaluation** – In this method, cells are segmented and tracked using the final outcome of every frame as an initial condition for the following frame [25]. These methods analyze individual frames or spatio-temporal volumes to create representations of object appearances or forms in order to track moving objects throughout time. In other words, it searches for similarities of objects in each image [47]. It is used where the object is not static, and the feature changes at any instance. This method is applied in applications like object tracking, edge detection, and handling cell division by providing a primary segmentation for the first frame [43]. Tracking by model evaluation has some drawbacks- firstly, low contrast edges or noise pollution leads to incorrect segmentation. Secondly, the sensitivity of models to the beginning position [44]. This method is divided into two types- parametric and non-parametric models.

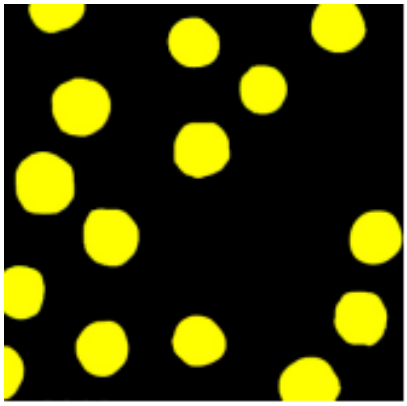


Fig. 3: Semantic Segmentation [9].

Fig. 4: Instance Segmentation [9].

- Parametric models – The energy function established by parametric models contain both internal and external energy conditions. These circumstances are brought about by the user's constraint pressure. They are easily identified as parametric contours and are frequently utilized in 2D applications. Therefore, with the exception of topological events like track dividing or merging, parametric models offer a quick and real-time implementation. These models are utilized to monitor a single object and to track the partial occlusion of objects and are capable of producing better estimates in cell morphologies [8].

- Non-parametric models – Non-parametric deformable models showcase a level set of scalar functions with higher dimensional that can handle the changes of topologies at a higher computational cost [46]. Re-initialization is necessary for the conditions of appearance and disappearance of cells from the field of view [8].

3) **Tracking by Filtering** – Tracking based on filtering is also known as Monte Carlo Technique [40]. This technique applies stochastic filtering techniques based on the implicit movement or appearance model of tracked objects [8]. The stochastic filtering technique is based on the tracked objects' fundamental movements or appearance patterns. This method is used in large displacements and broadly used in multiple object-tracking systems. The fundamental concept behind filtering techniques is to estimate the density function of the object's state posterior with the help of a set of random particles with connecting weights [8]. Filtering methods are branched into three stages- selection, prediction, and measurement that are performed iteratively.
In the selection step, a new set of particles is generated by choosing those from the previous particle set with the highest posterior probability.
The prediction stage involves changing each particle in the state model of the surrounding region of interest, and the subsequent measurement step involves reassigning each particle's weight based on the new information. A resampling step is necessary to escape the agglomeration of weight by good particles and raised the punishment of bad particles.
However, tracking is based on a particular feature of a large number of the proposed particle filter in a series of video sequences, whereas multiple-feature tracking provides a better explanation of the object and enhances the robustness of the method. It is important that any deformation of the tracked object is small, as large deformations cease their application to individual cell tracking [41,47,48]. Tracking by filtering can be divided into deterministic and statistical methods.

   - Deterministic method – This method can be used to connect new and past situations when the object's movement is limited and can be reliably predicted [49]. This technique takes the aid of a collection of motion limitations to create communication between each object in the previous frame with the single object in the present frame [50].
   - Statistical methods – This method is also called the probability method. These methods are used in situations where the state space approach is more effective in determining properties like position, speed, and acceleration [49]. Probabilistic methods take into account the object's measurement and indefiniteness while determining communication [50].

4) **Point-based Tracking using Spatial Context** – This method is used for estimating the positions of objects and their attributes jointly [53]-[56]. In this approach, an object is modeled as a single point which is the center point of the bounding box [54]. The detector uses keypoint estimation in order to find center points. CenterNet, a center point-based approach, is simpler, faster, and more accurate than corresponding bounding box detectors [54]. CenterNet approximates the center position heatmap and the size map, which stores the width and the height of the object's bounding box. The advantage of this method is their effective use of the spatial context as they directly extract common image features for position estimation and motion using a single network. However, a situation occurs where cells often partially overlap, and this situation continues for several frames. In order to avoid this difficulty, a cell tracking method is developed using long-term spatial temporal context [37].

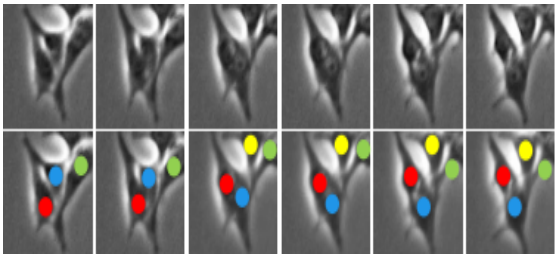


Fig. 5: Top: original images, Bottom: ground-truth of trajectories. From the 3rd frame, the blue and red cells touch and form a cluster, hence it is difficult to identify cells from the 3rd and the 4th frame. The individual cells can be identified if the entire frame is observed [37].

Tracking using long-term spatial temporal context – Using 3DCNN, this technique concurrently calculates the position and short-term and long-term motion of each cell in multiple frames, where long-term motion makes it easier to extract the long-term spatial-temporal context and is used for interpolating false negatives [37]. An object-level warping loss is carried out during the network's training to maintain consistency between the predicted motion and locations in several frames. This method performs tracking by object-level warping by transferring a region of each cell from $t-1$ to t by using the estimated motions.

III. Datasets for cell tracking

1) **Cell Tracking Challenge (CTC)** – CTC is a benchmark on publically available data for time-lapse cell segmentation and tracking. This is used to compare and evaluate state-of-the-art whole-cell and nucleus segmentation and tracking methods. The datasets include 2D and 3D time-lapse videos of fluorescently marked nuclei or cells moving on top of or within a substrate, together with 2D PhC (Phase Contrast) and DIC (Differential Interphase Contrast) microscopy videos of cells that are moving

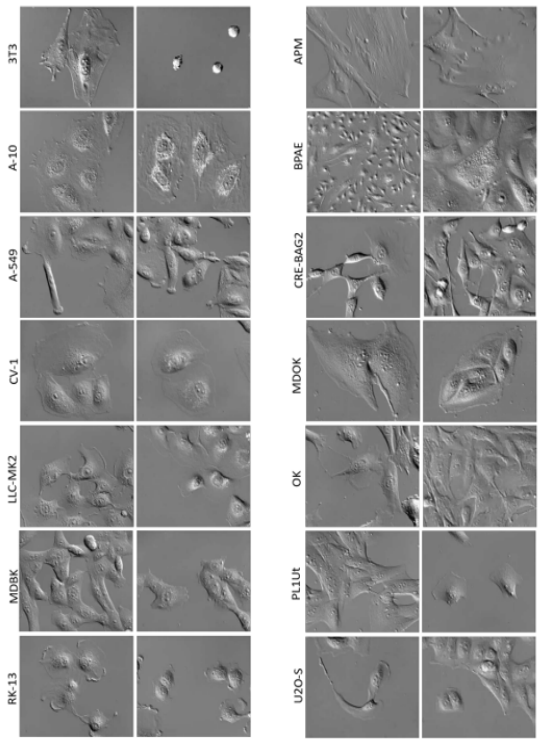
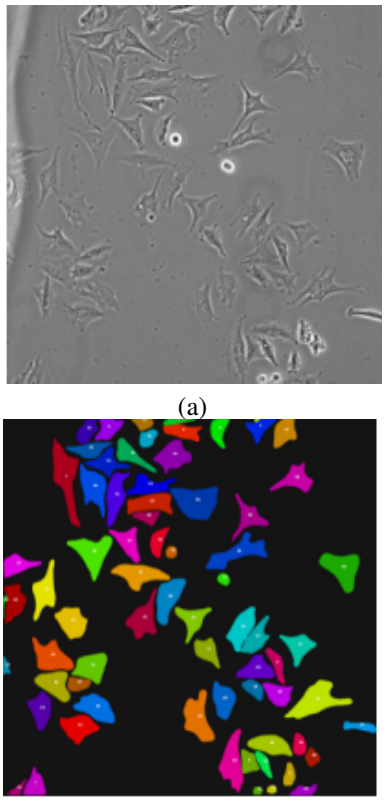


Fig. 6: Examples of frames for each of the 14 cell lines in CTMC dataset that depicts the diversity in cell morphology and frame density [61].

on a flat substrate [9]. The ground truth consists of cell markers connected together between frames to form cell lineage trees (for tracking) and manually annotated cell masks (for segmentation).

2) **DeepCell** – It comprises of a PhC image sequence of HeLa-S3 cells. The annotations are given in terms of cell and nuclei segmentation masks for each image [9].
3) **Usiigaci** – This includes 37 PhC images of T98G cells [9]. The annotations comprise indexed masks, with an index for each cell, followed in time. This dataset can be used for both segmentation and tracking [9].
4) The **dataset by Ker et al. [51]** comprises of 48 PhC image sequences of mouse C2C12 cells under various treatments. Additionally, the data comprises automatically generated annotations for every cell that was created using in-house software, which is based on segmentation, mitosis detection, and association.
5) **C2C12-16** – The first international competition on mitosis detection in phase-contrast microscopy image sequences uses this dataset for the mitosis detection task. This dataset is an extension of the Ker et al. dataset [51] with manual annotations of mitosis. This dataset comprises of 16 sequences of 1013 frames per sequence with a total of 7159 mitosis events within the images [9].
6) **Cell Tracking with Mitosis Detection Challenge (CTMC)** – This challenge provides DIC images for 14 cell lines [61]. This consists of 152,584 frames in total, adding up to 86 live-cell imaging videos [9]. The challenge also grants bounding box-based detection and tracking ground truths for each cell line in the form of CSV files for for each frame and each cell, the cell ID and its bounding box coordinates [9].



(a)

(b)

Fig. 7: Example data from the Usiigaci dataset [9] : (a) Original Image; (b) each color indicates a different cell in all sequence images for corresponding indexed masks

IV. Evaluation metrics for cell tracking

Metrics are used for the evaluation of the results quantitively. Some metrics are more specific to cellular image analysis, and the rest are directly used from the computer vision and Machine Learning/Deep Learning domains.

1) Jaccard similarity index, also known by Intersection over Union (IoU), determines the extent of overlap between the true and the computed results, given the ground truth cell segmentation GT and the corresponding segmentation S. It is defined as

$$IoU(GT, S) = \frac{|GT \cap S|}{|GT \cup S|} \tag{1}$$

where $|.|$ denotes the cardinality of the set, and $\cap$ and $\cup$ indicate the set intersection and union, respectively [9].

This is also expressed in terms of the number of true positive pixels TP ($TP = |GT \cap S|$), false negative pixels FN ($FN = GT\text{-}S$), and false positive pixels FP ($FP = S\text{-}GT$) [9]

$$IoU(GT, S) = \frac{TP}{FN + TP + FP} \tag{2}$$

Further, metrics can also be evaluated as-

- Recall, known as the True Positive Rate, computes the percentage of detected true positive pixels compared to the total number of true positive pixels in the ground truth [9].

$$Recall = \frac{TP}{TP + FN} \tag{3}$$

- Precision, known as Positive Prediction, gives the percentage of detected true positive pixels against the total number of pixels detected by the algorithm [9].

$$Precision = \frac{TP}{TP + FP} \tag{4}$$

- F-score- It is the weighted harmonic mean of Precision and Recall [9].

$$\begin{aligned} F - score &= \frac{2.Precision.Recall}{Recall + Precision} \\ &= \frac{2.TP}{2TP + FP + FN} \end{aligned} \tag{5}$$

All the above-mentioned metrics have their values in the range of [0,1]. The higher the value, the better the outcome.

2) Metrics for Object-Wise Cell Detection- The IoU metric of Equation is used to evaluate the degree of overlap between the ground truth bounding boxes (GT) and the predicted bounding boxes (S). IoU thresholding can then be used to decide whether the detection is correct. For a given IoU threshold α, a true positive (TP) is a detection for which the $IoU(GT, S) \geq \alpha$ and a false positive (FP) is a detection for which the $IoU(GT, S) < \alpha$. A false negative (FN) is an actual instance that could not be detected [9].

The metrics Recall, Precision, and F-score metrics defined in Equations (3) -- (5) can be used for the evaluation of cell detection algorithms. These are also used for the computation of the Average Precision at a given IoU threshold α, denoted as $AP@\alpha$, defined as the Area Under the Precision-Recall Curve (AUC-PR) evaluated at the IoU threshold α, given

$$AP@\alpha = \int_0^1 p(r)\, dr \tag{6}$$

Single values for α generally equal to 0.5 or 0.75 can be chosen for thresholding IoU. A set of thresholds has to be chosen and the mean Average Precision mAP over these IoU thresholds considered for cell detection evaluation [9].

$$mAP = \frac{AP\alpha 0.5 + AP\alpha 0.55 + ... + AP\alpha 0.95}{10} \tag{7}$$

with α from 0.5 to 0.95 with a step size of 0.05 DET, the detection accuracy of the methods, is adopted to estimate the identification accuracy of each given object. It is based on comparing the nodes of the acyclic-oriented graphs that represent the objects in the computed object detection result and the ground truth [9]. Exploiting the Acyclic Oriented Graph Matching measure for detection (AOGM-D) [58], which gives the cost of altering the set of nodes of the computed objects into the collection of ground truth nodes, DET is defined as

$$DET = 1 - \frac{min(AOGM - D, AOGM - D_0)}{AOGM - D_0} \tag{8}$$

where $AOGM - D_0$ is the cost of creating the set of ground truth nodes from scratch. DET always falls in the [0,1] interval, with higher values corresponding to better detection performance. The DET metric is averaged with the SEG metric to provide the overall performance of the CSB

$$OP_{CSB} = \frac{DET + SEG}{2} \tag{9}$$

3) Metrics for Cell Event Detection- Each detected mitosis is represented as a triple (x, y, t) of spatial and temporal position of the event in case of mitosis detection. The detection is considered to be true positive (TP) if its distance from the corresponding ground truth triple is below preset spatial and temporal thresholds else it is considered to be false positive (FP). Undetected ground truth mitotic events are considered false negative (FN). DeepCell is also used to perform semantic segmentation to segment individual cells and predict their cell type [4]. The Cellular Classification Score (CCSc) for each class c is considered for evaluating the results.

$$CCS_c = \frac{\sum_{i \in Cells} S_{i,c}}{\sum_{j \in Classes} \sum_{i \in Cells} S_{i,j}} \tag{10}$$

where $S_{i,j}$ indicates the classification score of pixel i for class j. The closer the CCS_c is to 1, the prediction is likely to be correct.

4) Metrics for Cell Tracking - The metrics opted for evaluating cell tracking are those introduced by Multiple Object Tracking (MOT) [60]. Multiple Object Tracking Accuracy (MOTA) [61] is a metric that represents object coverage. It is defined as:

$$MOTA = 1 - \frac{FN + FP + IDSW}{T} \tag{11}$$

where FN is the number of bounding boxes related to ground truth not covered by any computed bounding box, FP is the number of bounding boxes not covering ground truth bounding box, IDSW is the number of bounding boxes that enclose a ground truth bounding box from a track that is distinct from the previous frame, and T is the total number of detections in the ground truth.

Multiple Object Tracking Precision (MOTP) [62] is the average dissimilarity between all detections that are

TABLE I: Approaches used for different datasets

Datasets	Methods	Approaches
DeepCell	Tracking by model evaluation	Supervised Deep Learning [83]
CTMC	Tracking by detection	Human Annotation and automatic interpolation [61]
Usiigaci	Tracking by detection	Mask R-CNN [84]
C2C12-16	Point-based tracking	Spatial-Temporal coordinates [85]
Dataset by Ker et al.[51]	Tracking by detection	Segmentation and Association [51]
	Point-based detection	Manually Tagged Centroid [51]

correctly assigned (true positives) and their ground-truths. It is defined as:

$$MOTP = \frac{\sum_{t,i} d_{t,i}}{\sum_t c_t} \tag{12}$$

where c_t is the number of matches in frame t and $d_{t,i}$ is the bounding box overlap of the detection i with its ground truth. This MOT tracking measure demonstrates the tracker's ability to estimate precise object positions, irrespective of how well it can recognize object configurations or maintain consistent trajectories.

Recently, the MOT challenge introduced another tracking metric, named IDF1, that quantifies the object's identity across the frames of a sequence. It represents the ratio of correctly identified detections over the average groundtruth and computed detections [63]. It is an F-score equation like in (5)

$$IDF1 = \frac{2 * IDTP}{2 * IDTP + IDFN + IDFP} \tag{13}$$

where IDTP, IDFP, and IDFN depict the number of true positive, false positive, and false negative IDs, respectively.

Evaluation of multi-object tracking (MOT) has historically been challenging. Previous metrics overstate the significance of association or detection. MOTA is unable to precisely capture association. Higher Order Tracking Accuracy (HOTA), a revolutionary MOT evaluation metric explicitly balances the impact of carrying out accurate detection, association, and localization into a single unified statistic for comparing trackers [64]. The HOTA measure improves upon the MOTA metric (Multi-Object Tracking Accuracy) while addressing many of its drawbacks. The aims of HOTA are to (i) provide a single score for tracker evaluation that reasonably combines all different aspects of tracking evaluation; (ii) assess long-term higher-order tracking associations; and (iii) decompose into submetrics that permit analysis of the various performance-related elements of trackers [64].

$HOTA_\alpha$ score calculated at localisation threshold α is as follows:

$$HOTA_\alpha = \sqrt{\frac{\sum_{c \in TP} A(c)}{|TP| + |FN| + |FP|}} \tag{14}$$

$$A(c) = \frac{|TPA(c)|}{|TPA(c)| + |FNA(c)| + |FPA(c)|} \tag{15}$$

TPA, FNA, FPA, and *A(c)* depict True Positive Associations, False Negative Associations, False Positive Associations, and Asssociation score respectively.

IDF1 and Track-mAP, on the other hand, display counterintuitive detection performance. HOTA addresses these issues with a straightforward, elegant formulation that gives equal weight to detection and association accuracy [64].

The metric adopted in Cell Tracking Challenge for evaluating cell tracking results is the Tracking Accuracy, denoted as TRA [7]. It is defined as a normalized weighted distance between the tracking ground truth and the result of the algorithm, with weights to reflect the effort it takes for a human to manually carry out the edits needed to match the two. Tracking results are first represented as acyclic-oriented graphs providing the cells' lineage. Then the difficulty in transforming a computed tracking graph into the corresponding ground truth graph is estimated as [9]

$$TRA = 1 - \frac{min(ACGN, AOGM_0)}{AOGM_0} \tag{16}$$

where AOGM is the Acyclic Oriented Graph Matching (AOGM) measure and AOGM0 is the AOGM value to create the ground truth graph from scratch. The range of TRA is within [0,1], with higher values corresponding to better tracking performance. The performance for the CTB (Cell Tracking Benchmark) is computed as the average of the SEG and TRA metrics [9]

$$OP_{CTB} = \frac{SEG + TRA}{2} \tag{17}$$

V. Discussions

Table I summarizes the different approaches and methods for each dataset mentioned in section III.

In, DeepCell dataset tracking is considered a linear assignment problem. The idea behind DeepCell is that objects are unlikely to move large distances from frame to frame if the frame rate is high. A cost function is constructed for possible pairings across frames based on each object's location and appearance. A supervised deep learning approach is used to learn an optimal cost function for the framework of linear assignment. The method used in the DeepCell dataset is tracking by model evaluation [83].

The CTMC dataset uses bounding boxes for tracking by detection. A user draws a bounding box around the cell on the frame of its first occurrence in the video. The user readjusts the bounding box to cover the cell at any future frame of

their choice. The bounding box for the cell in the intermediate frames is interpolated using linear interpolation to reduce human workload. Mitosis events can be manually tracked by marking the cells with a flag when cell division occurs [61].

In Usiigaci, Phase Contrast Microscopy Images are processed in mask R-CNN segmentation module with ResNet-101 and the feature pyramid network to generate instance segmented masks. These instance-segmented masks are then linked and tracked using TrackPy based tracker which uses the k-dimensional tree algorithm [84].

The C2C12-16 dataset focuses on annotating events using point-based tracking. The spatial-temporal coordinate of each mitotic event in the microscopy image sequence was manually annotated with the form of (x,y,t). The coordinate indicates the time when a cell starts to split, and the location of the center of the two daughter cells [85].

The dataset by Ker et al. [51] uses point-based tracking as well as tracking by detection. Point-based tracking is done manually by individually tagging cells by placing a marker at the center of the cell. Computer-aided cell tracking uses tracking by detection where the cells are first segmented and then associated over consecutive frames.

VI. Conclusion

Computational object tracking techniques help identify cellular processes, cellular organs such as cytoplasm, plasma membrane, and nucleus and monitor mobility in consecutive frames. Some applications of cell tracking include finding barriers in the path to avoid overlapping, monitoring abnormality, and finding any defect in the cell maturity cycle. In some subcellular situations where high level of spatial resolution is needed, tracking systems produce satisfactory outcomes (e.g. the molecular dynamics of dendritic spines) [8].

There are many challenges in computational object tracking that have to be addressed and one of them is errors in tracking results. The key factors that cause error in tracking results [8] are:

- Noise- When images have a high noise ratio, it is difficult to detect objects. In some cases, the noise is followed instead of the objects and this happens when the noise level is almost equal to the object's intensity. Although there have been many solutions presented for noise reduction, most of them compromise a lot of true information that are occasionally valuable in order to remove more noises. As a result, there is a demand for solutions that are more effective while sacrificing less data [8].
- Information loss- Information loss occurs when converting 3D images to 2D. Further research can be done to lessen the consequences of this conversion utilizing novel techniques, such as automated cameras for the target item or determination of the proper camera position [8].

The common issue is the optimization of the algorithms' runtime and speed. The majority of tracking algorithms experience slower computation speeds as calculations become more complex, which lengthens run-time. In order to solve numerous biological challenges, such as deciphering complex cellular activities, it is necessary to create multitasking systems that can track several objects and simultaneously visualize the cell's environment.

Despite the fact that numerous object tracking algorithms have been developed over the past two decades, few of them have been applied in biological studies, and the majority of them have not yet reached their full potential. It is anticipated that additional studies on the use of in vitro experiments and in silico approaches will lead to new discoveries in this area [8].

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