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A survey on applications of deep learning in microscopy image analysis

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ABSTRACT

Advanced microscopy enables us to acquire quantities of time-lapse images to visualize the dynamic characteristics of tissues, cells or molecules. Microscopy images typically vary in signal-to-noise ratios and include a wealth of information which require multiple parameters and time-consuming iterative algorithms for processing. Precise analysis and statistical quantification are often needed for the understanding of the biological mechanisms underlying these dynamic image sequences, which has become a big challenge in the field. As deep learning technologies develop quickly, they have been applied in bioimage processing more and more frequently. Novel deep learning models based on convolution neural networks have been developed and illustrated to achieve inspiring outcomes. This review article introduces the applications of deep learning algorithms in microscopy image analysis, which include image classification, region segmentation, object tracking and super-resolution reconstruction. We also discuss the drawbacks of existing deep learning-based methods, especially on the challenges of training datasets acquisition and evaluation, and propose the potential solutions. Furthermore, the latest development of augmented intelligent microscopy that based on deep learning technology may lead to revolution in biomedical research.

1. Introduction

Microscopy plays an indispensable role in biomedical research. As the developments of optics and computer science, advanced technologies of microscopy have opened up a new eyesight for biomedical researchers. Phase contrast (PC) [1] and differential interference contrast (DIC) [2] microscopy are the most commonly used techniques to image living cells with transmitted light. They transfer the information encoded in the phase of the imaging filed into the intensity distribution of the final image, while atomic force and scanning electron microscopy are more suitable to render the 3D quantitative shape measurements of samples.

Fluorescence microscopy, such as confocal and total internal reflection fluorescence microscopy (TIRFM) have been widely used in biomedical research to observe subcellular structures with specific labeling. A vital limitation of conventional fluorescence microscope is that it cannot resolve subcellular structures below the diffraction limit, which is approximately one-half the wavelength of the excitation light (~200 nm) [3]. Super-resolution microscopy is a new trend of microscope development. It breaks the diffraction limit and records biological processes at the nanometer scale [3]. These new technologies enable us to acquire quantities of high-quality images, which contain assorted biomedical information. At the same time, we are confronted with new challenges of digesting these images by quantitatively processing the data. Therefore, using computational methods to augment the performance of microscopy and make it multifunctional in post-processing has become another fast-growing topic in the field.

Many traditional image processing methods, such as morphology, feature extraction, region growing, and etc. have been applied to analyze biological microscopy images. However, these methods routinely require computational experts who are uncommon among biomedical scientists [4] and involve sophisticated calculations. Besides,

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due to imaging constraints and diffraction limitation, conventional fluorescence microscopy images always have relatively low resolution and poor signal-to-noise ratio [5], for which traditional image analysis methods could not have a robust performance.

Deep learning, as a method of representational learning of data [6], is a branch of artificial intelligence. Although proposed as early as the 1940s, it has not been widely studied and used until 1980s. Nowadays, deep learning has developed and evolved in all aspects of scientific fields, especially in the field of image processing. Deep learning models such as Convolution Neural Network (CNN), Recurrent Neural Network (RNN), Generative Adversarial Nets (GANs) have achieved satisfactory results in many aspects, such as image classification, object detection, image segmentation, object tracking and super-resolution reconstruction [7].

Different from traditional image processing methods, one of the key advantages of deep learning is that its layers of features are not designed by humans. Instead, the features are learned from the data using a general-purpose learning procedure [6]. Specifically speaking, deep learning is able to learn from end to end itself [8] and do not require complex manual computation but result annotation instead. With the development of computer science, deep learning is able to deal with problems with quantities of parameters, and may have a higher speed, higher accuracy, and better robustness in complicated situations. In fact, many researchers are combining deep learning with traditional methods, using traditional methods for pre- or post-processing work, and deep learning for more difficult, computationally intensive tasks [9].

In the microscopy image analysis field, researchers are beginning to apply deep learning into many challenging problems. Most of these problems are concerned above cells, subcellular structures and tissues [5,10]. This review is organized in order of applications of deep learning in microscopy image analysis. We categorize them into four groups according to the main research targets. The overall taxonomy used in this literature is shown in Fig. 1 and Fig. 2. For each part, we begin the discussion with some popular networks which have been successfully applied to the corresponding target and followed by the detailed applications. Then we conclude the challenges and development for each subject.

Classification. Designing classifiers to identify different types of cells or cells at different stages of differentiation and recognize special cells or subcellular structures from other parts can be helpful for drug screening or disease diagnosis. It may assist doctors to diagnose a variety of diseases and predict the risk of cancer. In most applications, start with deep learning-based classifiers achieve higher accuracy and have faster processing speed than traditional classifiers.

Segmentation. Segmentation and localization of cells or any other regions that people are interested in is a crucial step in microscopy image analysis. It can help researchers focus on areas with rich and useful information from quantities of data. Intracellular compartments segmentation provides quantitative information about cell structure and function. Deep learning-based segmentation methods can be divided into semantic-level segmentation and instance-level segmentation based on whether if the framework contains a fully connected layer. Most of the semantic-level segmentation methods are based on U-Net, while instance-level segmentation algorithms derive from R–CNN. Studies have demonstrated that deep learning-based segmentation methods achieve higher accuracy than traditional methods. They have been applied for cell counting, morphometry analysis and tissue image analysis.

Tracking. Measuring the velocity of cells or intracellular targets overtime is essential to understand lots of meaningful biological signals. For example, the trajectory of the nuclei is related to cell localization and the dynamic information of the microtubule is associated with cell mitosis and vesicle transportation. The most commonly used networks for localization are based on RNN. With the development of Long Short-Term Memory (LSTM) and Attention Model, the problem of gradient vanishing and gradient exploding are solved. Previous researches have proposed several deep learning-based frameworks to analyze the cellular dynamics along a predictable trajectory, which is necessary for early disease diagnosis, evaluation of the drug effects, wound healing and neural crest migration.

Reconstruction. Image reconstruction is the creation of a two- or three-dimensional image from scattered or incomplete data. In microscopy image analysis, it includes denoising, super-resolution reconstruction and 3D reconstruction, which is important to acquire high-quality and informative images. Traditional methods to acquire images of high quality are limited due to expensive hardware and extensive post-imaging processing. Especially for single-molecule localization

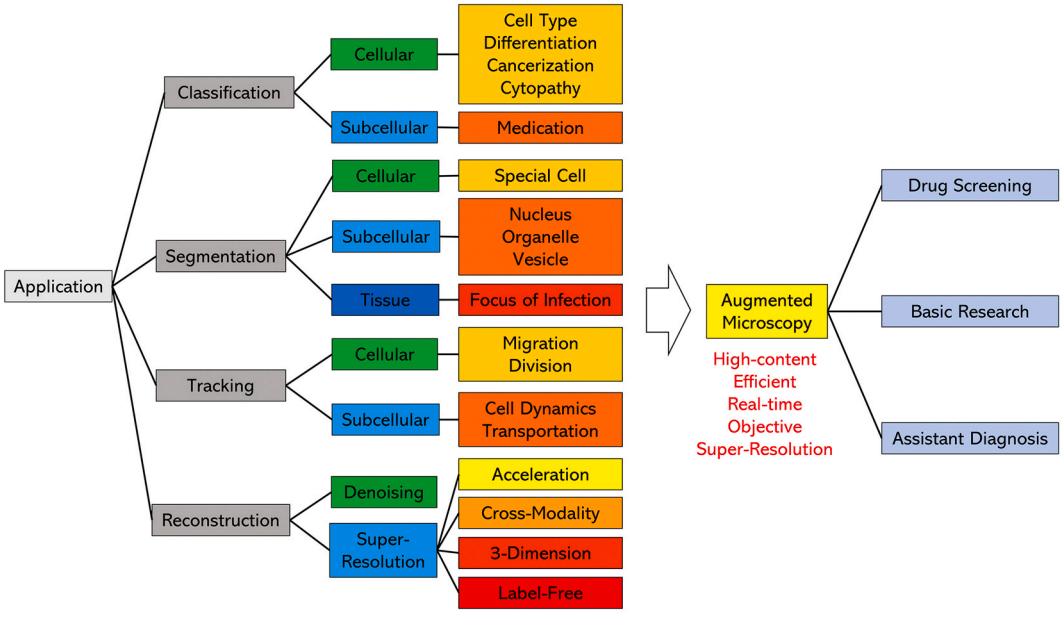

Fig. 1. Applications of deep learning in microscopy image analysis. The applications mainly include image classification, segmentation, tracking and reconstruction for different objects at varied scales. The augmented intelligent microscope that combines microscopy with deep learning is a promising concept, which would be of great significance for biomedical research.

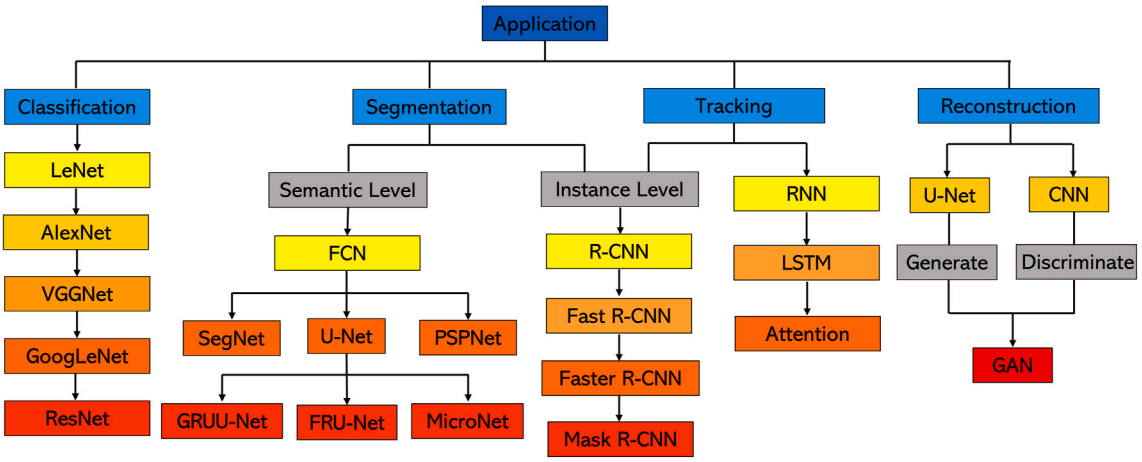

Fig. 2. Widely used networks for microscopy image analysis. Different networks have been developed to accommodate different tasks. The CNN for classification, FCN for segmentation, RNN for tracking and GANs for reconstruction. Each network has gone through long periods of evolution.

microscopy (SMLM), which needs to record thousands of frames to reconstruct a single super resolution image, it sacrifices temporal resolution for spatial resolution. Since the fluorescent labeled biological samples usually vary in quality, people would use different laser power, scanning speed and exposure time to meet the requirements. Deep learning-based image restoration methods can be used to capture light-sensitive samples with high speed and enable long-term dynamic observation of living cells with high quality. As for SMLM, CNN-based single-molecule localization models can be helpful to speed up the data-processing procedure with robustness. The U-Net and GANs have been used to enable super-resolution imaging across different microscope systems, which can be used for converting diffraction-limited input images to super-resolved ones [11].

Furthermore, intelligent augmented microscopy, as a promising concept, which combines microscopy with deep learning, enables super-resolution imaging and high-content, efficient, real-time analysis.

In all, in this review, we summarize the latest applications of deep learning in microscopy image analysis and their performance (Fig. 1). We also introduce some popular networks which have been successfully applied to microscopy image analysis (Fig. 2). Furthermore, we discuss the main challenges and future directions of deep learning in microscopy image analysis.

2. Classification

2.1. Deep learning-based classifiers

Image classification can be regarded as a task of assigning a label to an input image according to a certain rule. The labels usually come from a predefined set of possible categories. Commonly used image classification methods include Bayesian classifier, geometric classifier, clustering and neural network classifier. As deep learning develops, neural network classifiers, especially those based on convolution neural network (CNN), are growing in popularity.

Neural networks for image classification have been developed for a long time in other fields. Recently, in the field of microscopy imaging, many researchers have achieved inspiring results using deep learning-based classifiers. The commonly adopted framework is CNN and its derivative structures (Fig. 3). For a model trained from CNN, the input usually consists of different kinds of microscopy images, while the output is a vector containing the probability of each predefined label. Most of the models contain two parts, the feature extraction module consisting of convolution and pooling layers, and the classification module consisting of fully-connected layers. The numbers of the feature extraction layers and parameters depend on the complexity of the tasks. CNN classifiers have gone through a long time of development and

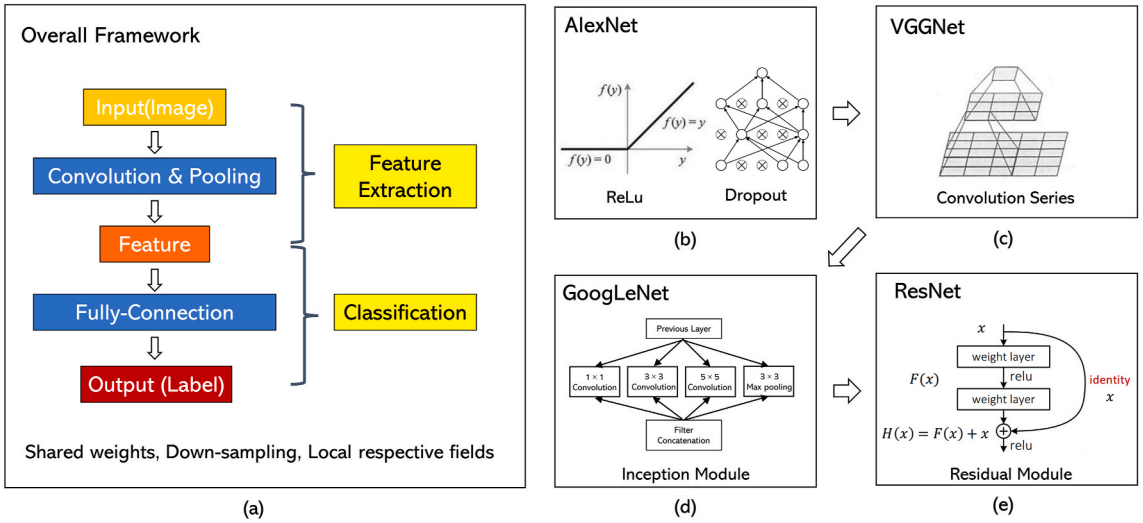

Fig. 3. Overall framework of CNN for classification and its evolution. (a) The overall framework for image classification, which contains a convolution layer and a fully-connection layer. **(b)–(e)** CNN has gone through a long period of evolution and has many derived structures.

evolution. LeNet [12] was firstly proposed as the overall framework for CNN image classifiers. Then AlexNet [13] introduced Rectified Liner Unit (ReLU) activation function and dropout to prevent gradient diffusion and overfitting. VggNet [14] was developed to increase network depth while reducing parameters by using convolution series instead of a big kernel size. GoogLeNet [15] proposed an inception module and increased model complexity without exponentially increasing network parameters. ResNet [16] introduced a residual module, which solves the problem of model degradation as the layer number increases. Furthermore, there're still many advanced CNNs that have been proposed beyond ResNet.

2.2. Applications of deep learning-based classifiers

Cellular and subcellular classification. Studies have demonstrated that without any human intervention and biological or hand-crafted features, deep learning-based classifiers were able to identify different types of cells [17] and cells at different stages of differentiation [18,19] with high accuracy. These deep learning-based methods also achieved satisfying performance on low-resolution images [20], where other classifiers always failed. Fluorescent images with cellular organelle staining, such as microtubule networks [21] and mitochondrial [22] have been used to train the classifiers, and these datasets can be used as a potential tool for drug screening on intracellular targets eventually.

Disease diagnosis. Another important function of deep learning-based classifiers is disease diagnosis. Researchers have proposed several classifiers to identify white blood cells [23–25]. Alzubaidi *et al* developed a classifier to recognize elongated (sickle) cells from normal red blood cells using CNN [26]. It may assist doctors to diagnose leukemia, anemia and other blood related diseases. Deep learning-based classifiers have also been used for facilitating the detection of other human diseases, such as autoimmune disease [27], cancer and so on. There have been deep learning models for the classification of lung cancer subtypes [28], diagnosis of hepatic granuloma [29], detection of breast cancer [30] and colon cancer [22]. A robust CNN model that can predict the risk of diseases according to cell analysis has also been proposed [31].

Performance. In most applications, deep learning-based classifiers achieve an accuracy(Jaccard index) of over 90% (see details in Table 1), which is much higher than traditional classifiers. In addition, they have faster processing speed than traditional ones, which makes high throughput detection possible. Studies have applied the classifiers on ultra-high-throughput microscopy systems and achieved efficient bulk classification for abnormal cellular morphology [32,33]. In addition, there are also combinations of deep learning-based classifiers with advanced light microscopy, such as SIM, to analyze viral structures [34].

2.3. Challenges and development of deep learning-based classifiers

Many previously established deep learning-based classifiers, such as LeNet, VGGNet and GoogLeNet could perform consistently on microscopic images by large data training or using transfer learning. Transfer learning is a popular method in training deep learning-based classifiers. Different from traditional learning procedures, transfer learning applies knowledge or patterns learned from one task to another different but related problem [35]. Studies have used a pre-trained CNN architecture to extract features from images and then fed them into a fully connected (FC) layer to realize a classifier which can distinguish normal cells and breast cancer cells [30]. By that, without any complicated parameters updating for the feature extraction layers but only for the FC layers, a smaller training dataset and minimized training effort could be achieved. Therefore, transfer learning provides deep learning with better transferability, economizes the datasets and allows researchers to deal with different but similar problems easily. Indeed, transfer learning has been applied widely in many cell classifying problems.

One of the major challenges of deep learning is that training a model requires a large number of labeled datasets. In some cases, the datasets are easy to obtain, while in other cases, it takes a lot of efforts to annotate ground truth images. Dataset augmentation strategy is a typical way to achieve efficient model training on a relative smaller dataset. Classic data augmentation methods include image flipping, image rotating, intensity altering, and so on [23,25].

Many researchers have used public datasets to train and test their models [22,23]. The easily accessible public datasets would help save manpower on data annotation and provide a unified evaluation index. However, there is still a lack of open-source public datasets in the field of microscopy image processing.

3. Segmentation

3.1. Deep learning-based segmentation methods

Image segmentation is the process of dividing an image into several regions with certain properties that people are interested in. Traditional segmentation methods include edge detection, threshold processing, region growing, morphology watershed algorithm, and so on. However, each of these methods has its own drawbacks. Deep learning has become a widely used method for image segmentation in every field. A CNN-based algorithm called Micro-Net was proposed for microscopy image segmentation [36]. It can process images with low signal to noise ratios (SNRs), variable intensities, complicated cellular structures. Image segmentation can be divided into semantic-level segmentation and instance-level segmentation (Fig. 4).

Semantic segmentation classifies each pixel in an image into the foreground and background. Fully Convolution Network (FCN) is a widely used network for semantic segmentation [37], which consists entirely of convolutional layers but no fully connected layers. Many popular models for segmentation, such as SegNet, PSPNet and U-Net are derived from FCN. Among them, the most widely used model in microscopy image segmentation is U-Net, which is named for its U-shaped network structure. U-Net can be divided into two processes: down-sampling and up-sampling [38,39]. The down-sampling is mainly realized by convolution and pooling layers, which extract image features, increase the size of the sensing field, ensure the robustness of model and decrease the risk of overfitting. The up-sampling is realized by deconvolution, which restores and decodes the abstract features. U-Net uses skip-connection to solve the problem that the original input data would gradually lose its characteristics as the network deepens. It has been successfully applied to segment cells in images with dense populations [40]. Furthermore, U-Net can be trained by using a small training dataset and can segment objects in microscopy images with strong robustness and high accuracy [38]. To date, many researchers have made improvements based on U-Net architecture to adapt to the requirements of their special tasks, for example, FRU-Net [41], Micro-Net [36], GRUU-Net [42]. Since the low contrast and irregular cell shapes in microscopy images will cause failures for U-Net, Zhao et al. developed a pyramid-based FCN framework to segment cells and obtain precise cell segmentation masks [43].

Instance-level segmentation is based on target detection, which identifies different objects in the image and classify them. The original model for target detection is called Region CNN (R–CNN). R–CNN extracts candidate regions that probably contain target objects by selective research, and then classifies each region and evaluates whether it contains the target by using CNN [44]. R–CNN takes significant time due to huge amount of computation. Therefore, Fast R–CNN [45] and Faster R–CNN have been developed based on R–CNN to increase the speed by tens or even hundreds of times. Furthermore, Mask R–CNN [46] was proposed to extend R–CNN to pixel-level detection, which enables instance segmentation.

Table 1
Deep learning algorithms and their performances in different tasks of microscopy image analysis.

Application	Network	Task	Public Dataset	Data Augmentation	Simulated Data	Accuracy
Classification	CNN	Breast cancer detection [30]	BreakHis	Yes	No	97.5%
	CNN	Cell type classification [20]	No	Yes	No	95%
	CNN	Non–small cell lung cancer classification and mutation prediction [28]	TCGA NYU Dataset	No	No	Classification 97% Prediction 73.3% ~85.6%
	CNN	C2C12 cells at differentiation classification [18]	No	Yes	No	91.3%
	CNN	Mice hepatic granuloma classification [29]	No	Yes	No	82.8%
	CNN	Red blood cell classification [26]	erythrocytesIDB	Yes	No	99.5%
	CNN	Cell classification of intracellular microtubule networks [21]	No	Yes	No	66%
	CNN	Cell classification of mitochondrial images [22]	No	No	No	98%
	Res-Net	Fine-grained leukocyte classification [24]	No	Yes	No	76.8%
	CNN	Stem cell multi-label classification [19]	No	Yes	No	87%
	CNN	White blood cells identification [25]	No	No	No	96.1%
	CNN	Acute Lymphocytic Leukemia detection in single cell blood smear images [53]	No	No	No	98.7%
Segmentation	U-Net	cell segmentation in microscopy images [154]	No	Yes	No	86%
	FCN	Mitochondria segmentation for EM data [61]	MiRA ATUM-SEM	Yes	No	90%
	HighRes3DZMNet	Mitochondria and endolysosomes segmentation for EM data [51]	UroCell dataset	Yes	No	90%
	SegNet	Blood Cell Images Segmentation [54]	ALL-IDB1 database	Yes	No	WBC 94.9% RBC 91.1%
	U-Net, Multi-Resolution Net	Cell segmentation of 2D phase-contrast microscopy images [48]	MDA-MB-231 dataset	Yes	No	89.1%
	FRU-Net	Small extracellular vesicles segmentation in EM images [41]	TEM image set of sEVs	Yes	No	Segmentation 62% Detection 75%
	U-Net	Image segmentation of dense cell populations [40]	DIC-C2DH-HeLa dataset	Yes	No	Segmentation 81.4% Detection 91.8%
	GRUU-Net	cell segmentation in microscopy images [42]	Cell Tracking Challenge	Yes	No	32.9%~93.8% on different datasets
	CNN	Vessel segmentation [62]	No	No	No	99.1%
	Micro-Net	segmentation of objects in microscopy images [36]	MICCAI 2017 CPMCC	Yes	No	83.5%
	FCN	Microvasculature segmentation [155]	No	No	No	99.1%
	U-Net	corneal endothelial cell images segmentation [56]	No	Yes	No	80%
	cGANs	Multi-organ nuclei segmentation in histopathology images	TCGA	No	No	86.6%
	U-Net, CNN	Mast cells segmentation and classification in histological images [64]	No	Yes	No	Segmentation 66.6% Classification 81.4%
Tracking	Faster R–CNN, U-Net	Cell tracking [156]	ISBI 2015 Cell Tracking Challenge	No	No	Mitosis Detection 81% ~91% Tracking 93.6%~98.7% Segmentation 64.8% ~87.2%
	CNN, RNN	submicron-scale particles [73]	No	No	Yes	95% on simulated data
	CTRL, U-NetR	dynamic measurement of single-cell volume [84]	No	Yes	No	94%
	ResCNN	Data association in cell tracking [93]	ISBI 2015 Cell Tracking Challenge	Yes	No	Tracking 95.5%–99.2% Segmentation 77.4%–91.8%
	DeepSeed (CNN)	Local graph matching for densely packed cells tracking [94]	No	Yes	No	Pair Detection 79% ~100%
	U-Net	cell segmentation, tracking, and lineage reconstruction [82]	No	Yes	No	Segmentation 83.7% ~99.9% Tracking 97%~99%
	CNN, LSTM	Instance-Level Microtubule Tracking [75]	No	No	Yes	Segmentation 68.1% Velocity estimation BVs 0.632
	Mask R–CNN	Nuclei Detection in Time Lapse Phase Images [74]	No	No	No	73.5%
	PCA-Net	Individual-cell tracking [79]	Mitocheck cell dataset	No	No	OPE Precision 58.5% ~70.7%
	CNN, TDNNs	Stem cell motion-tracking [83]	No	No	No	Normal cell 88.9% Mitotic cell 63.2%
	PCRM, PCOD (Faster R–CNN)	Identify Cell and Particle in Live-Cell Time-lapse Images [157]	No	No	No	Particle identification 90.2% Cell identification 99.9%

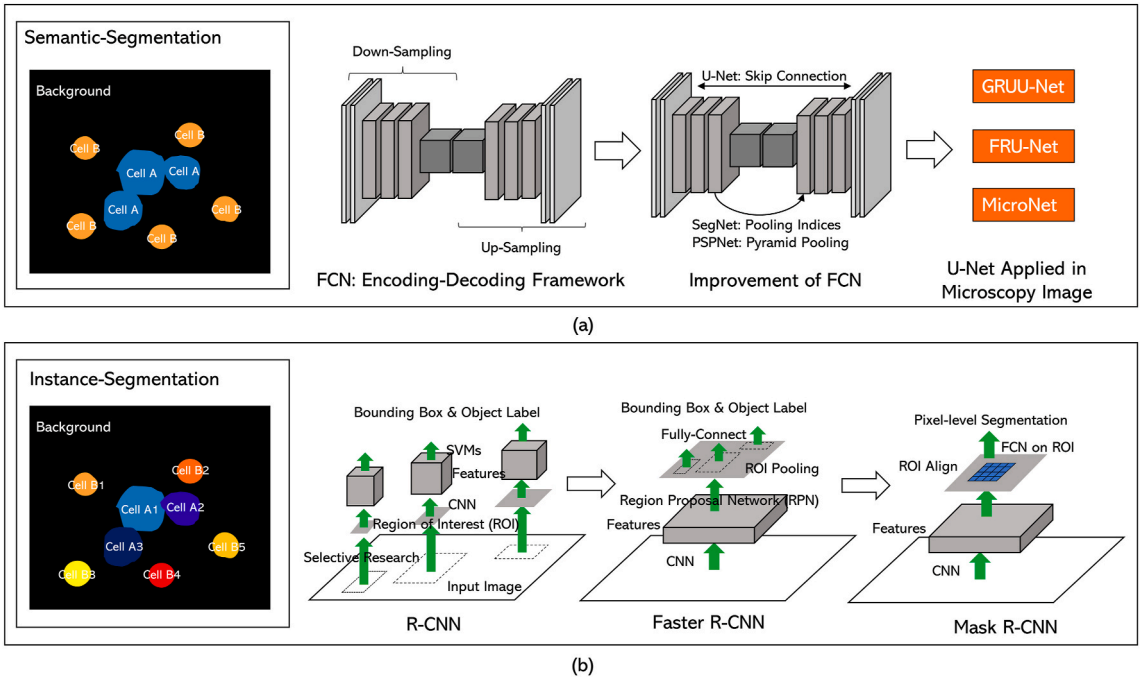

Fig. 4. Semantic segmentation, instance segmentation and their widely used networks. (a) FCN for semantic segmentation and its evolution. **(b)** R–CNN for instance segmentation and its evolution.

3.2. Applications of deep learning-based segmentation methods

Automatic and reliable characterization of cells is a crucial step in microscopy image analysis. Therefore, deep learning-based segmentation methods provide great convenience for microscopy image analysis. It can help researchers focus on regions of interest (ROIs) from quantities of data. Besides, it has been applied for cell counting [47] and cell structure analysis [48]. For example, Falk *et al* presented an ImageJ plugin based on a U-Net segmentation model, which can assist non-machine-learning researchers to analyze their data, including cell counting, detection and morphometry analysis [49]. There have been deep learning-based segmentation methods for different scales including cellular, subcellular and tissue scale.

Diagnose for diseases. Methods for high accurate cell detection and segmentation is greatly needed in drug discovery and cancer research. Nuh *et al* designed a computer-aided diagnosis (CAD) system based on deep learning, which can segment cancer cell patches from histopathological images and assist in cancer diagnosis [50]. Deep learning based segmentation algorithms have also been developed for detection of different cell types, such as breast cancer cells [51], liver cells [52], blood cells [53,54], cervical cells [55] and corneal endothelial cells [56].

Intracellular compartments segmentation. Besides segmentation on single cell scale, deep learning also performs well on the subcellular and tissue scale. Intracellular compartments segmentation provides quantitative information about cell structure and function [51]. One of the typical examples is cell nucleus segmentation, based on which multiple biological related analysis, such as cell type classification, cell counting and cell phenotype analysis can be performed [57,58]. A U-Net-based method was proposed for nucleus segmentation, which outperformed classical methods with improved accuracy and reduced the number of biologically relevant errors [59]. Also, a GANs based modeled has been applied on multi-organ nuclei segmentation in histopathology images [60]. Another similar task is organelles segmentation, whose morphology, number, distribution contain rich biological information too. Previous studies have proposed several segmentation models for mitochondria [51,61], endo-lysosome [51] and extracellular vesicles [41]. These techniques may provide the foundation for the basic research of organelle interaction, material transportation, intracellular metabolism and so on.

Tissue image analysis. In addition, segmentation methods based on deep learning also play an important role in tissue image analysis. Previous researches have proposed unique frameworks for vasculature network segmentation, which is important for early disease diagnosis and evaluation of the drug and hormone effects [62,63]. Karimov *et al* applied an architecture for the analysis of mast cells, an important player in the human immune system [64]. They detected mast cells from histopathological images sufficiently, which has a profound impact on various disease diagnosis and research.

3.3. Challenges and development of deep learning-based segmentation methods

Different from classification, the ground truth images for segmentation is much harder to annotate. The outline of the target object must be sketched, which is a very tedious and time-consuming process. For microscopy images with low resolution, it can be even more difficult to distinguish the edge of the objects. To date, although many open source databases, such as MoNuSeg [65] and TNBC Slides [66] have been provided to the public, it is still not enough to accommodate the needs. Haberl *et al* proposed and established a cloud-based deep convolutional neural network called CDeep3M to address this bottleneck, which generates training images to perform segmentation [67]. Yang *et al* proposed a new approach based on weakly supervised deep learning, which used box-annotations (to locate and enclose the targets using boxes) to train the model that alleviated the burden of manual annotation to a great extent [68]. Zhao *et al* proposed a weakly supervised training schemes to train end-to-end cell segmentation networks that only require a single point annotation per cell as the training label and generate a high-quality segmentation mask close to those fully supervised methods using mask annotation on cells [69]. In addition to simplifying the annotation process, data augmentation is another approach to deal with lack of datasets. Besides normal augmentation, there is a novel method called test-time augmentation (TTA) which

performs augmentation while training. This method includes four steps: augmentation, prediction, dis-augmentation and merging, and it achieved excellent results on semantic segmentation based on U-Net and instance segmentation based on Mask R–CNN [70].

4. Tracking

4.1. Deep learning-based object tracking

Object tracking is the task of following objects through a series of time-lapse images [4]. Developing object tracking methods to analyze the dynamic information in cell or subcellular structures are indispensable for cell biology research. Many algorithms have been developed for these purposes, such as Optical Flow method (OF), Fan-shaped Tracker (FsT) [71] and Kernelized Correlation Filter (KCF) [72], but most of them have their own limitations due to the special properties of microscopy images. Images with low SNRs and objects with unpredicted movements are the main challenges for traditional object tracking algorithm. In addition, frequent deformation, objects overlapping, random appearance and disappearance of the moving objects further make the targets hard to follow.

Object tracking tasks can be divided into two steps, the instance-level localization and the data association [73]. The most commonly used networks for localization are Mask R–CNN and RNN (Fig. 5). Mask R–CNN has been used to segment and track nucleus [74] and microtubule [75] in cell. Another widely used structure is RNN, whose output depends not only on the current input but also on the previous ones. RNN can preserve previous information and enable the model to memorize. However, in conventional RNN, the problem of gradient disappearance and gradient explosion in the process of long sequence training exists. To solve this, gating RNN has been proposed, of which Long Short-Term Memory (LSTM) [76] is a typical example. LSTM introduces a hidden state, which solves the problem of gradient vanishing and gradient exploding. This network acquires the ability to preserve useful information and discard unimportant ones so that the tracking accuracy and efficiency are greatly improved. So far, LSTM has been applied widely to object tracking in microscopy images [75,77,78].

4.2. Applications of deep learning-based object tracking in microscopy image

Cell tracking. Cell tracking is of great importance for basic researchers, to determine the drug treatment effects on cancer cells [79], to perform rapid antibiotic susceptibility testing [76], to analyze tumor cell metastasis [80], wound healing and neural crest migration [81]. The U-Net, which has been previously introduced for image segmentation, has also been used for cell tracking [82]. Wang *et al* proposed a CNN-based model that can track stem cells in microscopy images and detect mitosis with high accuracy [83]. A novel model called Cell Topography Reconstruction Learner (CTRL), which is developed based on U-Net, can measure single-cell volume over arbitrarily long time periods [84]. Mao *et al* solve the problem of mitosis event localization and its stage localization in time-lapse PC microscopy images using a three-step deep learning method with better performance than state-of-the-art [85]. Most cell tracking methods perform the association task independently from the detection task, however, a method called Motion and Position Map (MPM) that jointly represents both detection and association for not only migration but also cell division was proposed [86].

Intracellular particle tracking. Researchers have applied deep learning to analyze intracellular particle mobility and study the cellular dynamics [78]. A deep learning-based software for automated kymograph analysis called KymoButler was developed to visualize the dynamics of fluorescent particles, molecules, vesicles and organelles along a predictable trajectory [87]. Among different organelles, the nucleus that relates to cell localization and mitosis is of the most attention [74]. Many methods for nucleus detection and tracking have been proposed, including NucliTrack [88] and NucleiNet, which can track nucleus in time-lapse phase images. Another intracellular tracking target is microtubule, which is associated with cell mitosis and vesicle transportation [89]. An RNN-based tracker has been developed to track each microtubule instantly and measure its velocity over time [75] in microtubule gliding assay, to understand its dynamic regulation *in vitro* [90]. Li *et al* proposed an CNNs-based framework to quantitative analysis of vesicle-plasma membrane fusion events in the fluorescence microscopy, which is important in the vesicle exocytosis study [91].

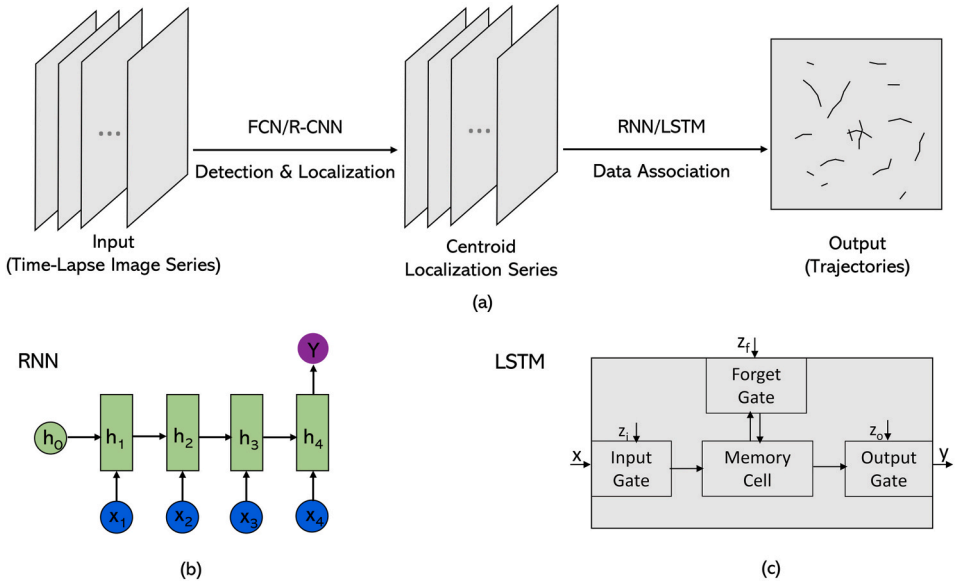

Fig. 5. Procedure of tracking task and the widely used RNN network. (a) The tracking task is often divided into the detection part and the association part. **(b)** The structure of RNN network. **(c)** The structure of LSTM network.

4.3. Challenges and development of deep learning-based object tracking

Combining the traditional linking algorithm with a deep learning-based detection method [92] and combining the traditional detection algorithm with a deep learning-based linkage model [93–95] are the most used solutions to improve the tracking performance. For multiple-target tracking, particle linkage between different frames after detection is another big challenge. Except for the U-Net mentioned earlier [82,90], some researchers proposed deep learning-based methods for particle linkage only. Since object linking can be seen as a classification task, they have used simple CNN and fully-connected layers to group the detected objects with the same trajectory. This method was proved to be effective for cell tracking [94]. Besides, there are also many other studies that used deep learning for objects detection and non-deep learning-based methods for linkage. Hungarian algorithm is a method for maximum matching in graph theory. Some studies have applied it to match the objects in adjacent frames and to track the stem cell [96] and microtubule dynamics [75]. Other mathematical methods such as Viterbi [97], template-matching [88] have also proved to be efficient.

The annotation of moving objects in microscopy images can be laborious and highly subjective. A crowd-based image-annotation platform called Quanti.us has been developed, which shown to increase 10–50 times in analysis efficiency compared with expert annotator [98]. Besides manually labeled datasets, scientists have chosen to use synthetic datasets, to simulate ground truth trajectories in time-lapse image sequences. The simulated data should share similar properties with real data, and they are used to pre-train the model and to select the hyper-parameters. Transfer learning method can be used to optimize the pre-trained model with annotated biological samples. Together, this will greatly reduce the amount of annotated data needed for model training. This strategy has been widely used in tracking of subcellular structures, which are hard to label manually on a large scale [72,75,78,88]. Besides, Li *et al* proposed a recommender system with correction propagation for debugging object tracking [99].

5. Reconstruction

5.1. Deep learning-based image reconstruction

Conventional super-resolution microscopy can be divided into two categories. One is based on the modification of point spread functions (PSFs), such as structured illumination microscopy (SIM) [100–102] and stimulated emission depletion microscopy (STED) [103,104]. The requirements of expensive optical modules and complicated mechanical parts have limited their universal adaptability. The other is based on single-molecule localization and complicated imaging analysis algorithm, such as photoactivated localization microscopy (PALM) [105], stochastic optical reconstruction microscopy (STORM) [106] and super-resolution radial fluctuations (SRRF) [107]. The imaging speed of these methods is limited by the need to record thousands of frames with a small number of observed molecules in each. Thus, it sacrifices temporal resolution for spatial resolution and is not suitable for live cells visualization.

Apart from traditional convolution network, GANs is a promising model for microscopy image reconstruction (Fig. 6). The GAN consists of two parts, a generator and a discriminator. The generator, usually an FCN or U-Net, is used to generate image and the discriminator, usually a CNN, is used to discriminate whether the generated image is real or fake. Through continuous gaming, the system will reach a balance where the generator can output synthesized images that the discriminator cannot distinguish. The GANs has been widely used in microscopy image denoising, super-resolution reconstruction and cross-modality translation and it enables weekly-supervised or unsupervised learning. However, GANs are dangerous too because of their potential to create artificial structure which are not present in the actual images [108], which is quite remarkable.

5.2. Applications of deep learning-based reconstruction in microscopy image

Optimization of the imaging parameters, image denoising and image restoration. The fluorescent labeled biological samples usually varies in quality, thus one would use different laser power, scanning speed and exposure time to meet the requirements. Optimization of these parameters is a time-consuming process that depends on advanced experiences. Deep learning networks have been used to automatically set the parameters for imaging, which enable the imaging system to be adaptive [109]. In addition, deep learning methods have been developed for image denoising [39,110]. These methods even outperforms several classical digital image denoising algorithms such as nonlocal means (NLM) [111] and deconvolution [112–114]. For example, the

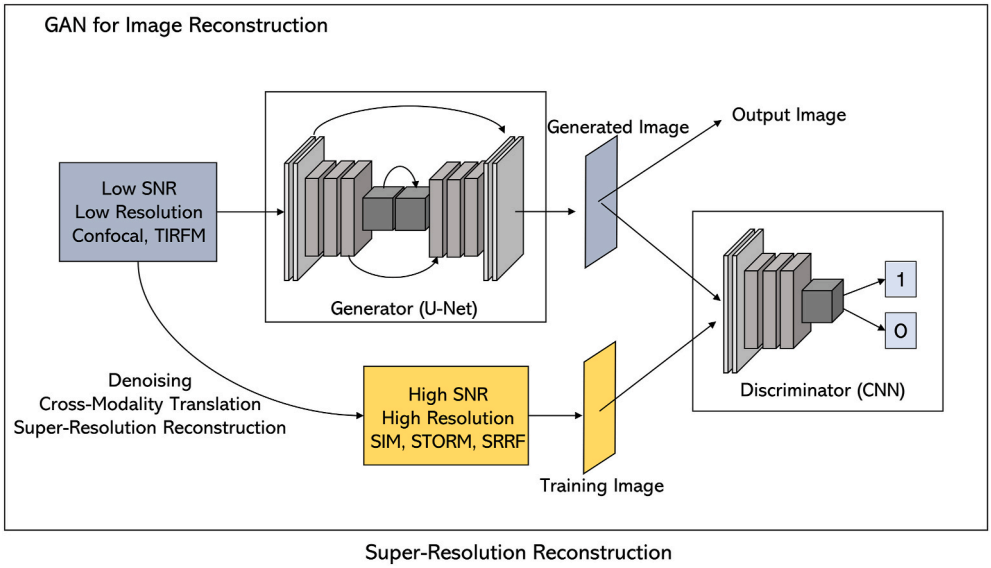

Fig. 6. GANs for image reconstruction in microscopy image. Training of GANs doesn't rely on matching of input and output data, which makes it popular in researches of image denoising, cross-modality translation and super-resolution reconstruction.

content-aware image restoration (CARE) network [39] was trained with low SNR input image and high SNR ground truth image pairs, which is capable of restoring images with 60-fold fewer photons in high quality. Therefore, image restoration methods based on deep learning algorithms can be used to capture light-sensitive samples with high speed and enable long-term dynamic observation of living cells with high quality.

Acceleration of imaging speed. As mentioned earlier, single-molecule localization microscopy (SMLM) sacrifices temporal resolution for spatial resolution and has limitation on visualization of live cells. Although several localization methods have tried to accelerate the data-processing speed, they still require a time-consuming iterative procedure and sample dependent parameter tuning [115]. CNN-based single-molecule localization models can be helpful to speed up the data-processing procedure with robustness. For example, Deep-STORM [116] was trained with a low-resolution input image and super-resolved ground truth image pairs. The ground truth image was reconstructed by the ThunderSTORM plugin in ImageJ [117–119]. Some researchers have tried to use the CNN with a small amount of PALM/STORM raw data to augment the performance of SMLM. One of the successful examples is ANNA-PALM [120], which is based on a special conditional generative adversarial network (cGAN) [121]. ANNA-PALM can reconstruct super-resolution images from sparse, rapidly acquired localization images and widefield images. A similar method is used in accelerating SRRF based super-resolution reconstruction. Using simulation images, previous work obtained a 20-fold reduction in the number of raw images required for SRRF reconstruction [107]. Furthermore, a U-Net-based network has also been used to augment the performance of SIM imaging with fewer raw images and dimmer light conditions, which increases the imaging speed by five times and allows long-term imaging in live cell [122]. They applied this methods on reconstruction of F-actin, mitochondria, adhesion and microtubule fluorescence images using transfer learning [122].

Cross-modality super-resolution reconstruction. Applications of traditional super-resolution fluorescence microscopy are limited due to expensive hardware and extensive post-imaging processing. The GANs has been used to enable super-resolution imaging across different microscope systems [110]. It can be used for image-to-image 'translation', that is, converting diffraction-limited input images to super-resolved ones. For instance, it has been shown to transform confocal microscopy images to match the resolution acquired with a STED microscopy or to transform total internal reflection fluorescence (TIRF) microscopy images to match the resolution acquired with a TIRF-based SIM microscopy. This cross-modality super-resolution method could serve to rapidly output super-resolved images, without any iterations or parameter search. It would be possible to achieve long-term super--resolution observation of living cells without any delicate hardware devices or harsh imaging conditions. In addition, PC and DIC are two noninvasive techniques for monitoring live cells. Algorithms based on deep learning have the capability of transferring images from one modality to the other modality for different requirements [123].

5.3. Challenges and development of deep learning-based image reconstruction

Many image analysis workflows perform 3D reconstruction of subcellular structures in order to extract meaningful biological information. One way to acquire 3D fluorescence information is by mechanical scanning through the sample volume to obtain images at multiple focal planes, such as confocal [124], two-photon [125], light-sheet [126,127] and other various super-resolution microscopy techniques [103,128–132]. However, the point scanning would limit the imaging speed and potentially introduce phototoxicity in cells. As for non-scanning 3D reconstruction methods, multi-angle total internal reflection fluorescence microscopy (MA-TIRFM) has gained a lot of attention as it has the potential to reconstruct the z-dimensional information [133–138], but the time-consuming iterative procedures and poor robustness limit its applications. To overcome these challenges, several artificial neural networks are trained to make a direct link between two-dimensional (2D) images and 3D super-resolution reconstructed results [139–141]. A framework termed Deep-Z is trained using GAN. It has the ability to rapidly refocus a 2D fluorescence image onto user-defined 3D surfaces [139]. The input 2D fluorescence image is first appended with a user-defined digital propagation matrix to represent images at the different axial locations, while the ground truth 3D fluorescence information is acquired through scanning through the sample volume to obtain images at multiple focal planes. After training with the Deep-Z model, one can image the activity of the living sample in 3D using a time sequence of fluorescence images acquired at a single focal plane, digitally increasing the depth-of-field by 20-fold without any axial scanning [139].

The PSF obtained from a fluorescence microscope is rich in information, such as the axial location embedded in the defocused PSF, on which the 3D-SMLM was based [142,143]. Zelger *et al* trained a CNN network to perform ultrafast 3D-STORM reconstruction [144]. The gold standard images in the training dataset were obtained by maximum likelihood estimation (MLE) [143]. Although the 3-D localization accuracy of MLE is great, it is noise-sensitive and susceptibility to ill-chosen initial values. While using the CNN network, it obtained similar localization accuracy but had a better stability. Furthermore, a machine learning-based 3D multi-color SMLM was developed. In addition to the axial location, the emission wavelengths of a fluorophore also sets the scale of PSF in all three dimensions [145]. According to the different shape information of fluorophore with different emission wavelength, a color-separating artificial neural network (ANN) with a final Softmax layer was trained using cross-entropy loss to determine the emitter color of each PSF. In parallel, ANNs for resolving the axial position of the emitter were separately trained for each fluorophore using L2 loss so that the final output was a scalar value [146] corresponding to the decoded axial position. Finally, an end-to-end framework from raw, noisy PSF images to the molecule characteristics is constructed, which is used to do multi-color 3D-SMLM reconstruction.

All the above-mentioned methods rely on the use of fluorescence labels. However, labeling is time-consuming and some specialized reagents can be toxic, preventing their usage in live cells. Researchers have developed a label-free method for predicting three-dimensional fluorescent information directly from transmitted light images and demonstrated that it can be used to generate multi-structure, integrated images [147]. In addition, a computational machine-learning approach termed as "in silico labeling" (ISL) was constructed. It can be used to reliably predict the fluorescent labels at different organelles in different cell types from unlabeled transmitted-light images [148], which further demonstrates the potential application of deep learning networks on super-resolution imaging.

6. Conclusion and outlooking

Deep learning technologies have been used widely in all scientific fields. For microscopy imaging, the acquired images always have variable SNRs, which contain rich information and require complicated computation [149], where traditional analysis methods are not sufficient. What's more, using traditional methods routinely requires experts on mathematics or programming, who are uncommon among biomedical scientists [4,150]. By contract, deep learning can deal with problems involving thousands of parameters and it has shown to perform with better robustness, higher speed and accuracy [151].

Till now, deep learning has been applied to microscopy image classification, segmentation, tracking and super-resolution reconstruction tasks. For different tasks, different models have been developed (see details in Table 1).

Although deep learning has shown its great advantages in microscopy image analysis, there are still some challenges remaining to be

solved. One of the most prominent drawbacks of deep learning is that a huge quantity of annotated datasets is needed for the training process, which requires tedious work and often introduces biases. Many researchers have started to share their data on public platforms, which is helpful to develop a unified evaluation index (see details in Table 1). However, there is still a lack of open source public datasets for microscopy image analysis. Data augmentation that increases the amount of data by flipping, rotating, intensity altering on the basis of existing data, and transfer learning which applies knowledge learned from one task to another different task have facilitated the establishment of reliable training datasets. GANs are used for data augmentation as well [108] despite of their potential of creating fake regions. In tracking tasks, synthetic datasets are always used in pretraining step, which could combine with transfer learning to reach better performance. The major obstacle to apply deep learning for super-resolution reconstruction is the need for matched training pairs of low-resolution input images and high-resolution ground truth data. Using simulation images for pre-training or applying an appropriate image alignment method to enlarge training dataset might be helpful for alleviation of the constraints on training datasets. Deep learning has achieved satisfactory results in classification and segmentation tasks. However, for particle tracking in microscopy images, especially for densely decorated multiple-target tracking, the performance of deep learning is still not sufficient. Novel models with higher accuracy and better robustness still remain to be developed. For instance, algorithms with trajectory prediction and abnormal instance detection are needed for these purposes.

Another dark side of deep learning is its poor interpretability. Although many mathematics and computer scientists are trying to explain the principle, deep learning is still function as a dark box [152, 153]. Especially in the medical field, researchers are also concerned with the facticity, privacy of the data, while the application of deep learning may carry unpredictable risks [108].

However, applying deep learning to microscopy has demonstrated to allow biologists to retrieve and reconstruct high resolution images without sophisticated hardware setups and complicated labeling and imaging conditions. Deep learning can only learn from existing knowledge and still has limitations on update and transfer knowledge. It usually requires large amounts of data and the trained models are not flexible and cannot handle multitasking. As deep learning technologies and microscopy techniques develop, an intelligent augmented microscope combining advanced microscopy techniques with deep learning may become possible, which would be equipped with super-resolution imaging and high-content, efficient, real-time, objective image analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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