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AN ANALYSIS OF VOLUMETRIC MICROSCOPY IMAGES FOR AUTOMATIC CELL SEGMENTATION USING DEEP LEARNING TECHNIQUES

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ABSTRACT

Volumetric microscopy images need to be subdivided into individual cells to analyse different biological processes. One of the most important imaging techniques in modern cell biology is three-dimensional electron microscopy. However, the identification of intracellular structures is a tedious and time-consuming task that hinders the efficient use of a potentially useful technology. Reducing this latency is a crucial next step in state-of-the-art biomedical imaging. Deep-learning methods for automatic segmentation have recently gained popularity due to machine learning's extensive success in bio-image informatics. Convolution neural network-based deep learning techniques have been created and demonstrated with impressive results. The application of deep learning techniques to processing microscope images, including high-resolution reconstruction, object tracking, region segmentation, and picture classification. Furthermore, let us examine the shortcomings of the existing deep learning-based systems, particularly the difficulties in locating and evaluating training datasets, and offer some recommendations

for possible remedies. Separating cell bodies, membranes, and nuclei from microscope pictures is necessary for many biological applications. This work offers a general overview of these works to highlight the challenges these works pose, outline earlier research methodologies and areas of study, and ultimately recommend future research directions.

Keywords: Deep learning, Convolutional Neural Network, Cell Counting, Cell Segmentation, Volumetric Microscopic Images, Cell Tracking.

1. Introduction

One of the primary areas of study in medical imaging is microscopy image cell segmentation, which focuses on analysing the geometrical shape, size, and other morphological characteristics of biological cells in addition to other duties, including cell identification, segmentation, and counting. In recent years, there has been a focus on automating the analysis of microscope image cells, initially with traditional image processing techniques. Consequently, the state-of-the-art automation method for a number of microscopy image cell tasks, such as microscopy image cell segmentation [12], has been greatly advanced by deep neural networks (DNNs), more especially encoder-decoder architectures. Previous research used DNNs to train a fully supervised cell segmentation model. Still, much microscope image data had to be gathered and labelled at the pixel level to make this model work.

Few-shot microscopy image cell segmentation is a technique recently demonstrated in a more useful study. It can be taught using a support set with several annotated microscope training images. This configuration involves training a deep neural network model with source data that includes training pictures from different kinds of cell segmentation issues. After that, a support set of a small number of randomly chosen and annotated microscope images is used to fine-tune the trained model to the target images with cells of interest. Even if random image selection is effective, it can still be made better because low informativeness in the support set could lead to poor fine-tuning and low accuracy performance in the testing target images that aren’t visible.

1.1 3D IMAGES

3D images, or three-dimensional images, are visual representations that convey depth perception, allowing objects or scenes to appear as if they have depth and volume. Unlike traditional two-dimensional (2D) images [11], which are flat and lack depth perception, 3D images give the illusion of depth, making them

appear more lifelike and realistic. There are various techniques to create 3D images, including stereoscopy, where two slightly different images are presented to each eye to simulate depth perception, and computer-generated imagery (CGI), where 3D models are created and rendered using specialised software. 3D images are commonly used in fields such as entertainment (movies, video games), medical imaging, architecture, virtual reality, and more, where depth perception adds value to the visualisation of complex structures or environments.

1.2 Volumetric Microscopy Images

Volumetric or 3D microscopy images provide detailed visualisations of biological samples or materials in three dimensions. Traditional microscopy typically produces 2D images, capturing a single plane of the sample at a time. However, with advancements in microscopy techniques, capturing entire volumes of samples has become possible, allowing for more comprehensive analysis and understanding.

Volumetric microscopy techniques include:

- **Confocal microscopy:** This technique uses a focused laser beam to illuminate specific planes within a sample. By scanning through different depths and collecting fluorescence emitted from the sample, confocal microscopy generates high-resolution 3D images.

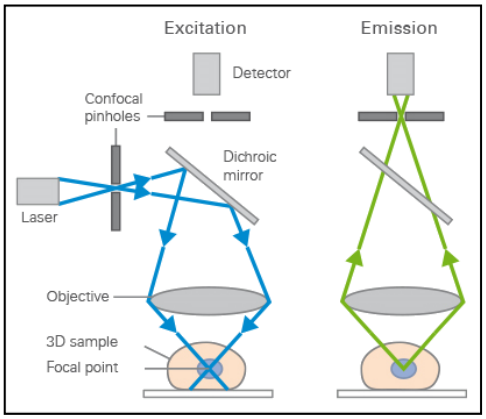

Fig 1: Confocal Microscopy

- **Two-photon microscopy:** Two-photon microscopy uses two lower energy photons to excite fluorescent molecules within a sample, allowing for deeper imaging without damaging the specimen. It is particularly useful for imaging thick samples, such as brain tissue.

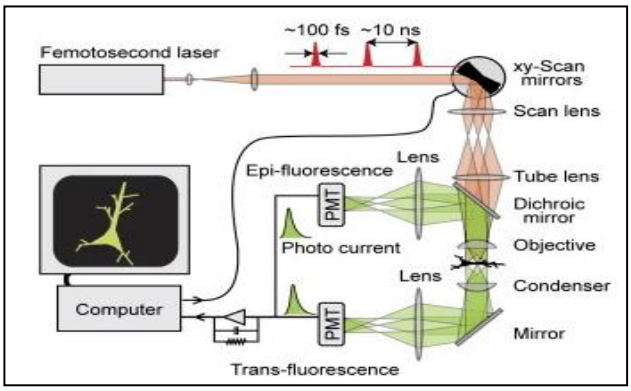

Fig 2 Two-photon microscopy

- **Light-sheet microscopy:** Light-sheet microscopy [14] illuminates a thin plane of a sample with a sheet of laser light while capturing images from a perpendicular angle. This technique reduces phototoxicity and allows for rapid imaging of large samples, making it suitable for live-cell imaging.

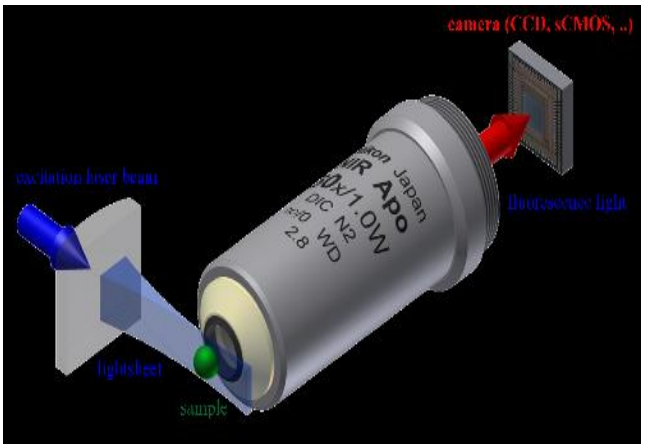

Fig 3: Light Sheet microscopy

- **Serial block-face imaging:** In this technique, a sample is imaged layer by layer as it is gradually shaved or sectioned. Each slice is imaged, and the process is repeated until the entire volume is captured. This method is often used for imaging tissues at nanometer-scale resolution in electron microscopy.

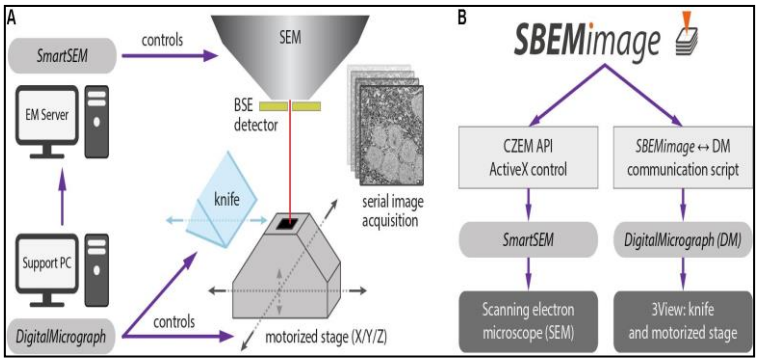

Fig 4: Serial block-face imaging

1.3 Microscopy Image Cell Segmentation

Microscopy image cell segmentation identifies and delineates individual cells within a microscopy image. It is a crucial step in quantitative analysis and allows researchers to study various aspects of cellular morphology, behaviour, and interactions.

Here's how the process generally works:

- **Preprocessing:** The microscopy image may undergo preprocessing steps to enhance contrast, reduce noise, and improve the quality of the image. This could involve techniques like filtering, background subtraction, or intensity normalisation.
- **Feature extraction:** Features such as cell edges, textures, or intensity variations are extracted from the preprocessed image. These features help distinguish cells from the background and each other.
- **Segmentation:** Segmentation algorithms are applied to partition the image into regions corresponding to individual cells. Various segmentation methods include thresholding, region-growing, watershed transformation, active contours (snakes), and machine learning-based approaches like convolutional neural networks (CNNs).
- **Thresholding:** Pixels with intensities above or below a certain threshold are classified as belonging to cells or background, respectively.
- **Region-growing:** Starting from seed points, neighbouring pixels with similar properties (e.g., intensity) are iteratively added to the segmented region until a stopping criterion is met.
- **Watershed transformation:** This method treats intensity gradients in the image as a topographic surface and simulates flooding to delineate cell boundaries.
- **Machine learning-based approaches:** Deep learning techniques, particularly CNNs, have shown promising results in segmenting microscopy images by learning hierarchical features directly from the data.
- **Post-processing:** The segmented regions may undergo post-processing steps to refine the segmentation and remove any artefacts or errors. This could involve morphological operations (e.g., erosion, dilation), connected component analysis, or manual correction.
- **Quantitative analysis:** Once the cells are segmented, various quantitative measurements can be extracted, such as cell count, size, shape, intensity, texture, and spatial distribution. These measurements provide insights into cellular behaviour, physiology, and pathology.

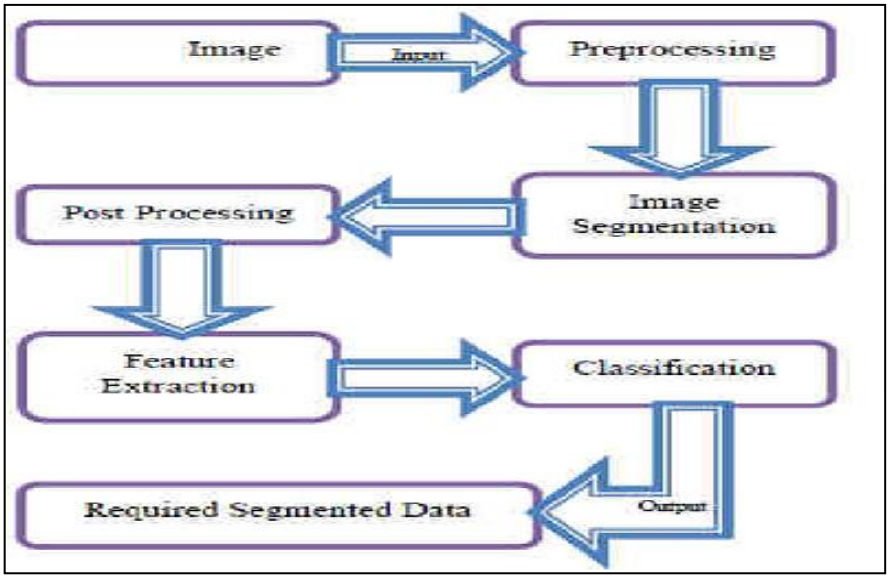

Fig 5: Flowchart for Image Cell Segmentation

2. Literature Review

Some of the research papers reviewed are given below:

S.no	Year	Name of The Paper	Author	Proposed Work	Explanation	Research Gap
1	2021	EM-net: Deep learning for electron microscopy image segmentation	Afshin Khadangi et.al.[1]	EM – NET	EM-net variants perform better than current deep learning methods using small- and medium-sized ground-truth datasets. We also show that the ensemble of top EM-net base classifiers outperforms other methods across various evaluation metrics.	EM-net applications can be extended to other tasks, including 3D volume segmentation, and we are quite confident that the proposed TLU would provide good results for other imaging modalities.

2	2021	FusionNet: A Deep, Fully Residual Convolutional Neural Network for Image Segmentation in Connectomics	Tran Minh Quan, et. al. [2]	FusionNet	FusionNet combines recent advances in machine learning, such as semantic segmentation and residual neural networks, with summation-based skip connections. This results in a much deeper network architecture and improves segmentation accuracy.	FusionNet is trained as a single chained network. More in-depth analyses into why chaining approaches are beneficial to improve the prediction accuracy of such deep networks will be an important goal for future work.
3	2021	Learning cellular morphology with neural networks	Philipp J. Schubert et.al. [3]	They had introduced cellular morphology neural networks (CMNs)	It was based on multi-view projections sampled from automatically reconstructed cellular fragments of arbitrary size and shape.	To learn about this cellular morphology with neural networks and some real-time datasets.
4	2023	MorphoFeatures for unsupervised exploration of cell types, tissues, and organs in volume electron microscopy	Valentyna Zinchenko et. al. [4]	A novel unsupervised method	For learning cellular morphology features directly from 3D EM data, a neural network delivers a representation of cells by shape and ultrastructure.	Feature Analysis of 3D EM will be done better.

5	2023	Understanding important features of deep learning models for segmentation of high-resolution transmission electron microscopy images	James P. Horwath et al. [5]	A novel method	They proposed methods for optimising image segmentation performance using convolutional neural networks, critically examining the application of complex deep learning models in favour of motivating intentional process design.	This method will also be applied to the challenging datasets in the future.
6	2022	Knowing What to Label for Few Shot Microscopy Image Cell Segmentation	Youssef Dawoud et. al. [6]	They proposed a new approach to optimise the image selection process.	They proposed novel self-supervised pretext tasks to compute the scores of unlabelled target images. Finally, the top few images with the least consistency scores are added to the support set for Oracle (i.e., expert) annotation and later used to fine-tune the model to the target images	This work needs to be extended by combining other selection techniques, such as diversity-based selection. Also, we plan to combine semi-supervised learning with support set fine-tuning.

7	2022	MEDIAR: Harmony of Data-Centric and Model-Centric for Multi-Modality Microscopy	Gihun Lee et. al. [7]	MEDIAR	MEDIAR is a holistic pipeline for cell instance segmentation under multi-modality in this challenge. MEDIAR harmonises data-centric and model-centric approaches as the learning and inference strategies, achieving a 0.9067 F1-score at the validation phase while satisfying the time budget	The MEDIAR framework does not use unlabeled datasets; how to properly incorporate approaches for unlabeled datasets would be a promising extension for MEDIAR.
8	2016	SAU-Net: A Unified Network for Cell Counting in 2D and 3D Microscopy Images	Yue Guo et. al. [8]	SAU-Net	They First proposed SAU-Net for cell counting by extending the segmentation network U-Net with a Self-Attention module. Second, we design an extension of Batch Normalization (BN) to facilitate the training process for small datasets.	Semi-supervised learning utilises unlabeled data jointly with labelled data to facilitate training. Both directions aim to take advantage of unlabeled data or data from other sources, effectively minimising the need for labels and could lead to further improvement.

9	2022	EfficientCell Seg: Efficient Volumetric Cell Segmentation Using Context-Aware Pseudocoloring	Royden Wagner et al. [9]	ViTs	Our model is efficient and has an asymmetric encoder-decoder structure with very few parameters in the decoder. Training efficiency is further improved via transfer learning.	In the future, we will have to try this using challenging datasets.
10	2021	Cellpose: a generalist algorithm for cellular segmentation	Carsen Stringer, et. al. [10]	Cellpose	It can precisely segment cells from various image types and does not require model retraining or parameter adjustments.	Periodically retraining the model on the community-contributed data will ensure that Cellpose improves constantly.

3. Methodology

3.1 Cell Counting

Cell counting in volumetric microscopy images quantifies the number of cells in a three-dimensional volume captured by a microscope. Unlike traditional 2D microscopy images, volumetric microscopy images provide information about cell distribution and density in a 3D space, which can be crucial for various biological and medical applications.

Here's how cell counting in volumetric microscopy images is typically performed:

1. **Pre-processing:** In automatic segmentation for cell counting in volumetric microscopic images, preprocessing is critical in enhancing image quality and preparing the data for accurate segmentation. Here's a breakdown of the preprocessing steps typically involved:
2. **Image Acquisition:** Volumetric microscopic images are initially captured using imaging equipment such as confocal microscopes or other

similar devices. These images comprise a stack of 2D slices [13], representing different depths or planes within the specimen.

3. **Noise Reduction:** Microscopic images often contain noise, which can interfere with segmentation accuracy. Noise reduction techniques such as Gaussian smoothing, median filtering, or wavelet denoising are applied to reduce noise while preserving important image features.
4. **Intensity Normalization:** Variations in illumination across the image stack can affect segmentation results. Intensity normalisation techniques are used to standardise the brightness and contrast levels across all slices in the stack, ensuring uniform illumination.
5. **Image Registration:** Volumetric images may sometimes suffer slight misalignments or distortions between slices due to specimen movement or imaging artifacts. Image registration methods align the slices properly, ensuring spatial consistency throughout the stack.
6. **Contrast Enhancement:** To improve the visibility of cell boundaries and other important structures, contrast enhancement techniques such as histogram equalisation or adaptive contrast stretching may be applied. These methods help to increase the visual clarity of the image stack.
7. **Background Subtraction:** In many cases, uneven background intensity across the image stack may interfere with segmentation accuracy. Background subtraction methods are utilised to remove or reduce the influence of background signals, focusing segmentation efforts on the foreground objects of interest (cells).
8. **Thresholding:** Once the preprocessing steps have been completed, thresholding is applied to segment the cells from the background. This involves selecting an appropriate intensity threshold to distinguish between foreground (cells) and background regions in each image slice.
9. **Morphological Operations:** After thresholding, morphological operations such as erosion, dilation, and smoothing may refine the segmented cell regions, remove noise, and fill gaps or holes within cell boundaries.
10. **Connected Component Analysis:** Connected component analysis is then applied to identify individual cells based on the segmented regions. This involves labelling connected sets of pixels belonging to the same cell and extracting relevant features such as cell size, shape, and intensity.

11. **Segmentation:** Cell segmentation delineates individual cells in the volumetric images from the background. This can be achieved using various image segmentation techniques, including manual delineation, thresholding, watershed segmentation, region growing, or more advanced deep learning-based segmentation methods [15].
12. **Cell Identification:** Once the cells are segmented, they are typically labelled or assigned unique identifiers to distinguish them from each other. This step is crucial for accurate cell counting and analysis.
13. **Cell Counting:** After segmentation and identification, the number of cells in the volumetric image can be determined by simply counting the labelled cells. This can be done manually or automatically using computational algorithms.
14. **Validation and Quality Control:** It's essential to validate the accuracy of the cell counting results, especially when using automated methods. This can involve comparing the automated counts with manual counts performed by experts or evaluating the consistency of counts across different volume regions.
15. **Analysis and Visualization:** Once the cell counts are obtained, further analysis can be performed to study various characteristics such as cell density, spatial distribution, and morphological features. Visualisation techniques such as 3D rendering, volumetric rendering, and statistical plots can aid in interpreting the results.

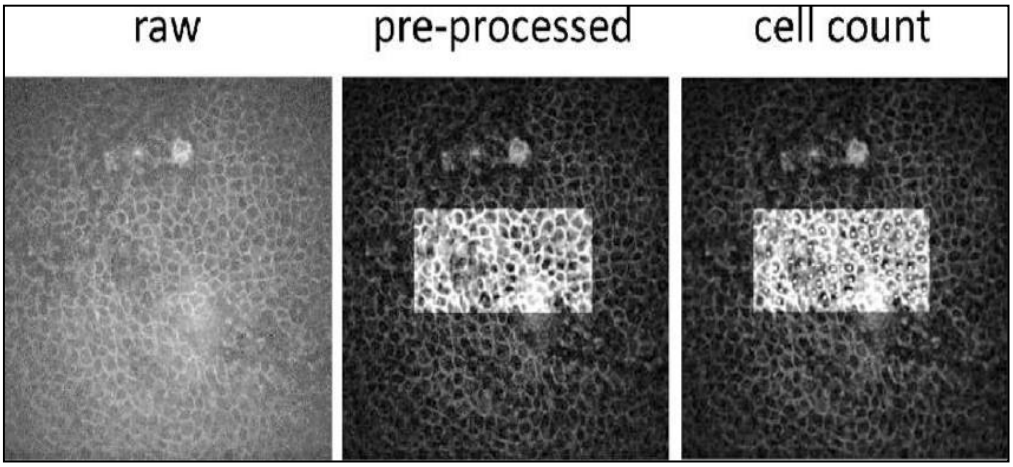

Fig 6: Cell Counting method

3.2 Few Shot Segmentation

Few-shot segmentation in microscopy images [20] refers to segmenting objects or regions of interest with limited annotated data. In traditional segmentation tasks, a large amount of labelled data is typically required to train accurate segmentation models. However, when obtaining such annotations [16] is expensive or time-consuming, few-shot segmentation methods aim to generalise well with only a few annotated examples.

In microscopy images, few-shot segmentation techniques are particularly valuable due to the often intricate and complex structures present, such as cell nuclei, organelles, or tissue structures. Here's how few-shot segmentation in microscopy images is typically approached:

- **Data Augmentation:** Since the labelled data is scarce, data augmentation techniques are often employed to artificially increase the diversity of the training dataset. Augmentation techniques such as rotation, scaling, flipping, and elastic transformations can help improve the model's generalisation ability.
- **Transfer Learning:** Pre-trained models can be fine-tuned for few-shot segmentation tasks, especially those trained on large-scale datasets like ImageNet. The model can effectively adapt to the target microscopy images with limited annotations by leveraging features from a diverse dataset.
- **Meta-Learning:** Meta-learning approaches, such as metric-based or optimization-based methods, are designed to quickly adapt to new tasks with limited data. Metric-based meta-learning is designed to adapt segmentation tasks by leveraging a metric or distance function to compare and learn from labelled examples. Here's how it works:

1. **Task Definition:** The segmentation task and its requirements are initially defined. This includes specifying the type of images to be segmented, the classes or objects of interest within the images, and any specific segmentation challenges or constraints.
2. **Dataset Preparation:** A dataset containing diverse labelled examples is prepared. This dataset typically consists of images and corresponding ground truth segmentation masks, where each pixel or region is labelled according to its class or category.
3. **Feature Representation:** Each image and its corresponding segmentation mask are converted into a suitable feature representation. This step involves extracting relevant features

from the images, such as pixel intensities, texture descriptors, or deep learning embeddings, which capture important information for segmentation.

4. **Metric Learning:** Metric learning is employed to learn a distance function or similarity measure between pairs of feature representations. The goal is to optimise this distance function to map similar examples closely in the feature space while dissimilar examples are pushed further apart.
5. **Meta-learning Setup:** The dataset is partitioned into meta-training and meta-test sets. The meta-training set is used to train the metric-based meta-learning model. In contrast, the meta-test set evaluates its performance and adaptability to new segmentation tasks.
6. **Training:** During the meta-training phase, the model learns to adapt its segmentation strategy based on the labelled examples provided in the meta-training set. This adaptation involves adjusting the parameters of the distance function or other model components to effectively segment images from the same distribution as the meta-training data.
7. **Adaptation:** Once the model is trained, it can be deployed to adapt to new segmentation tasks. When presented with a new image for segmentation, the model compares its features to those of the labelled examples in the meta-training set using the learned distance function. Based on this comparison, the model adjusts its segmentation strategy to produce accurate segmentation results for the new task.
8. **Evaluation:** The performance of the adapted segmentation model is evaluated on the meta-test set to assess its generalisation capabilities and effectiveness in handling new segmentation tasks. This evaluation helps to validate the utility of the metric-based meta-learning approach for segmentation adaptation.

- **Generative Models:** Generative models, such as generative adversarial networks (GANs) or variational autoencoders (VAEs), can generate synthetic training data that closely resemble the target microscopy images. Combining synthetic data generation with real annotated data allows the model to segment objects with fewer annotations accurately.
- **Active Learning:** Active learning strategies can be employed to select the most informative samples for annotation, thereby maximising the effectiveness of the limited annotation budget. By iteratively selecting samples for annotation based on the model's

uncertainty or informativeness, the model can perform better with fewer annotations.

Few-shot segmentation in microscopy images is an active area of research, with ongoing efforts to develop more effective algorithms and techniques to tackle this challenging task. Researchers aim to enable accurate and efficient segmentation of microscopy images with limited annotated data by leveraging advances in deep learning, transfer learning, and meta-learning.

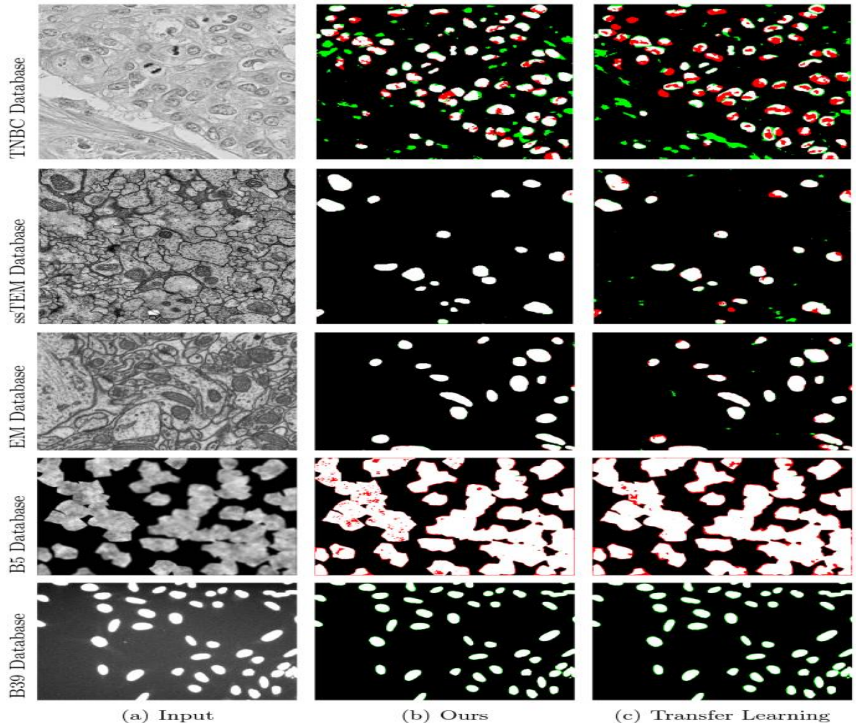

Fig 7: Few-Shot Microscopy Image Cell Segmentation

3.3 Deep Learning Techniques

Deep learning techniques offer powerful solutions for automatic cell segmentation in volumetric microscopic images. Here are some commonly used techniques:

- **Convolutional Neural Networks (CNNs):** The majority of deep learning-based image segmentation tasks rely heavily on CNNs. They learn hierarchical features from images and are particularly effective for tasks like cell segmentation. Architectures like U-Net, Mask R-CNN, and their variants are commonly used for this purpose.

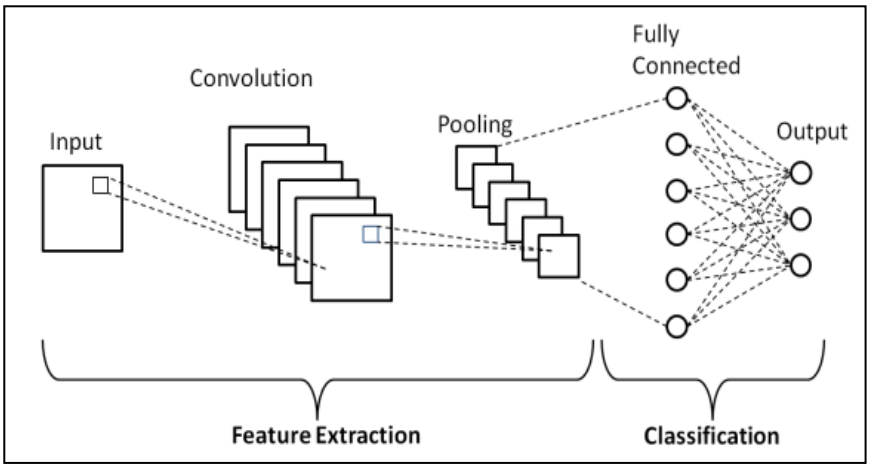

Fig 7: Convolutional Neural Networks

- **U-Net:** U-Net is a popular architecture for biomedical image segmentation tasks. It comprises a symmetric expanding path that allows for exact localisation and a contracting path that captures context. It has been widely used for volumetric image segmentation tasks due to its effectiveness and efficiency.

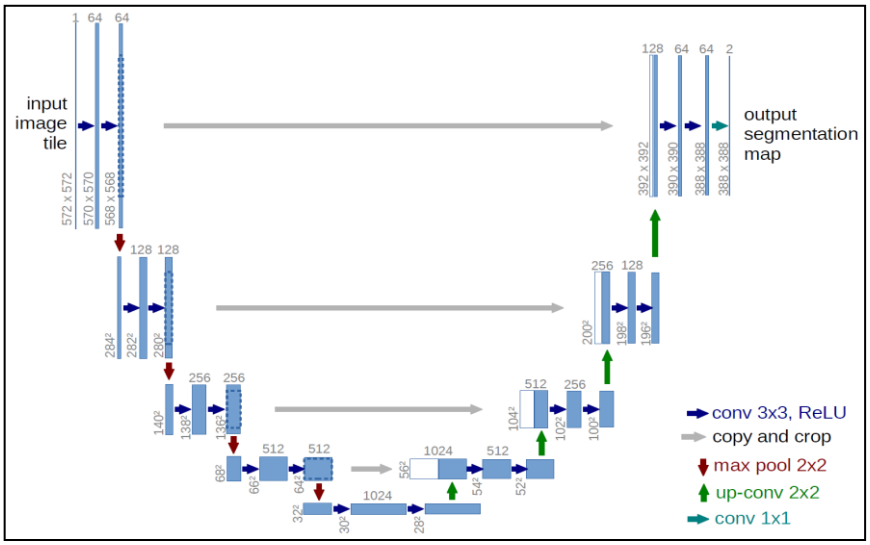

Fig 8: U-NET: Convolutional Networks

- **3D Convolutional Neural Networks:** Unlike traditional CNNs that process 2D images, 3D CNNs operate directly on volumetric data. They can capture spatial dependencies in all three dimensions, which is crucial for volumetric image segmentation tasks. However, they are more computationally expensive and require more memory than 2D CNNs.

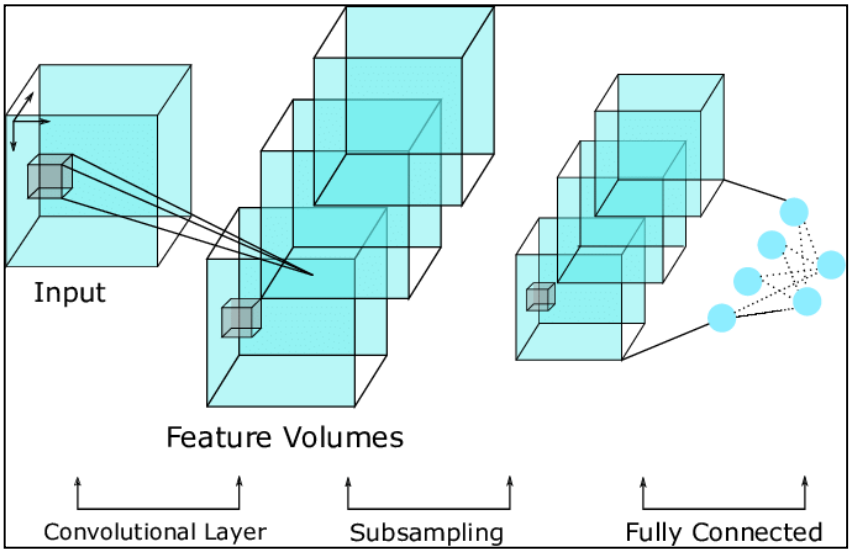

Fig 9: Basic 3D CNN Architecture

- **Attention Mechanisms:** Attention mechanisms[17] can help the model focus on relevant regions of the input volume, improving segmentation accuracy. They have been integrated into various architectures to enhance performance, especially when cells might be closely packed or irregularly shaped.
- **Data Augmentation:** Augmenting [18] The training data with transformations such as rotation, scaling, and flipping can help improve the robustness of the segmentation model and prevent overfitting, especially when dealing with limited training data.
- **Transfer Learning:** Pre-trained models trained on large datasets like ImageNet [19] can be fine-tuned for cell segmentation tasks. Transfer learning can help speed up training and improve performance, especially when limited annotated data is available.
- **Post-processing Techniques:** After obtaining initial segmentation masks from the model, post-processing techniques such as morphological operations (e.g., erosion, dilation), connected component analysis, and conditional random fields can be applied to refine the segmentation results and remove artifacts.
- **Ensemble Methods:** Combining predictions from multiple segmentation models trained with different architectures or initialisation parameters can yield more accurate and robust segmentation results than a single model.

By employing these deep-learning techniques, researchers and practitioners can develop robust and accurate automatic

cell segmentation systems for volumetric microscopic images.

Conclusion

Volumetric microscopy images provide researchers with valuable insights into biological specimens' 3D structure, organisation, and dynamics, aiding developmental biology, neuroscience, and cell biology. They enable the visualisation of intricate details that may not be apparent in 2D images, leading to a deeper understanding of biological processes. This review provides an overview of these works to summarise previous methods and research topics, highlight the issues raised by these works, and suggest future research directions. This paper's contributions will help researchers understand past developments and propose further innovative technologies.

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