

Quantitative Cell Analysis: Modern Approaches to Cell Counting, Process, Study

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Abstract

Cell counting is crucial in various fields, including biological research, biochemistry, and medical diagnostics. Traditional manual methods are labor-intensive, prone to errors, and can bottleneck workflows. This research explores using deep learning and machine learning for automated cell counting. The focus is on accurate segmentation and counting of cells in images. Convolutional Neural Networks (CNNs) are a key technique, alongside other sophisticated algorithms like Watershed, YOLO, Snake, and Otsu's method. The study also investigates hybrid approaches that combine these methods to enhance counting accuracy and reliability. By analyzing existing research and current trends, the study identifies key challenges and proposes future research directions to improve automated cell counting systems. These advancements have the potential to significantly improve efficiency in clinical and research settings by providing faster and more reliable cell counting solutions.

Keywords: Cell Image Segmentation, Digital Image Processing, Automatic Cell Counting , Biomedical images

Introduction and Motivation

Real-time computer systems are revolutionizing various sectors, especially medical research. Accurate diagnosis and treatment of diseases depend heavily on monitoring cells. Manual cell counting methods are laborious and limit the ability to provide optimal care. Our bodies are composed of distinct tissues and cells. However, some diseases, like cancer, pose treatment challenges. Cancer cells can evolve over time and in response to therapies, potentially leading to relapse[1]. An automated system capable of identifying and monitoring both healthy and abnormal disease-related cells could significantly improve treatment outcomes and prevent recurrence.

Cell counting is a crucial aspect of numerous biological applications, including:

- **Genetics:** Understanding genetic factors in diseases requires cell counting.
- **Biochemistry:** Cell counting is vital for studying cellular processes and their response to various stimuli.
- **Medical Diagnostics:** Cell counts like white blood cell counts are essential for diagnosing infections and other medical conditions. Different white blood cell types, such as neutrophils and lymphocytes, target specific pathogens.
- **Treatments:** Accurate cell counts are necessary for various treatment procedures, including cell transfection and cryopreservation (freezing for later use). Monitoring cell health and growth rates is also crucial.

The Need for Speed and Accuracy

Cell counting needs to be fast, precise, and consistent for reliable analysis of cellular responses. This is why automated cell counting techniques are being developed. These techniques aim to overcome the limitations of manual methods and improve efficiency in various research fields.

Future Considerations

The text mentions tables (Table 1 and 2) that likely address specific research questions and relevant acronyms related to cell counting. These details could be incorporated into a more comprehensive report for a deeper understanding of the field.

Table 1 lists the research questions this Cell Counting survey addressed.

Sr.no	Question	Answer
1	What is the time duration of this survey?	1960–2024
2	What are the important techniques for cell counting?	Four: Spectrophotometers, Flow Cytometers, Coulter counters, and Hemocytometers
3	Which segmentation algorithms	Four: region-based, pixel-based, boundary-based, and histogram-based
4	What's new in cell imaging research?	Segment cell mitosis, morphometry, counting, kinetics, and dynamic interactions between cells
5	Present Difficulties with Cell Counting	Four: Adhesion, complicated borders, irregular corona, varied physiognomy, and semantic gap
6	Recommended Systems for Accurate Cell Counting	Hybrid of soft computing, such as neural networks, with algorithmic techniques, such as snake and watershed
7	Essential Steps in Cell Counting Systems	Three: Counting, detecting, and segmenting cells

Table 2: Definitions of acronyms

Sr.no	Acronym	Definition
1	CIS	Cell-image Segmentation
2	ANN	Artificial Neural Networks
3	PBS	Pixel Based Segmentation
4	HBS	Histogram based segmentation
5	CIN	Cervical Inter-epithelial Neoplasia
6	LLM	Local Linear Map
7	ML	Machine Learning
8	fMRI	Functional Magnetic Resonance Imaging

Layout of the Article

1. Background and Introduction: The basic ideas of cell biology, such as the many cell kinds and their architecture, are covered in this part. Additionally, an overview of cell image segmentation is given, along with an explanation of its significance in relation to cell counting. The context for comprehending the requirements and intricacies of automated cell counting systems is provided in this section.

2. Literature Review: This section examines a variety of cell counting strategies, emphasizing both conventional and cutting-edge approaches. It gives an overview of the most latest advancements in the discipline, with a special emphasis on techniques for counting cells from microscopic pictures. To give a thorough grasp of the present state of research, important studies and their conclusions are reviewed.

3. Cell Counting Process: There are three stages. Cell detection, counting, identification of cell regions, and post processing have been discussed.

4. Different Methods for Cell Counting: This section covers four different methods for counting cells, each with pros and cons of their own. We investigate strategies including watershed, YOLO, hybrid approaches, and convolutional neural networks (CNNs). We explore the usefulness and practical uses of various methods, offering suggestions for how they may be put into practice.

5. Difficulties with Image Processing and Cell Counting: The third part lists and describes the ongoing difficulties with image processing that are connected to cell counting. Problems including picture quality, different cell sizes,

and cell overlap are looked at. It emphasizes how much better algorithms and methods are required to increase accuracy and dependability.

6. Future Scope and Research Directions: This section addresses the several possible uses of cell counting in research, therapy monitoring, and medical diagnostics. Prospective avenues for study are suggested, with an emphasis on using cutting-edge technology to surmount existing constraints. The section emphasises how crucial it is to keep coming up with new ideas in order to advance image processing and automated cell counting.

1. Overview and Background

An important test that physicians regularly request to evaluate a patient's condition is a complete blood cell count (CBC) [3,4,5]. Blood consists of white blood cells (WBCs), red blood cells (RBCs), and platelets as its primary components. Red blood cells, also known as erythrocytes, make up 40–45% of total blood cells. Blood also contains a significant amount of platelets, also referred to as thrombocytes. WBCs, also known as leukocytes, account for just around 1% of total blood cells[6,7]. The number of red blood cells directly influences the amount of oxygen they transport to the body's tissues. RBCs carry oxygen to the body's tissues. WBCs combat infections, and platelets help in blood coagulation. The traditional manual hemocytometer method for counting blood cells is slow and prone to errors due to the large quantity of blood cells. The accuracy of the method largely relies on the skills of the clinical laboratory analyst performing the count [8,9]. Therefore, utilizing an automated technique would significantly assist in counting various blood cells from a smear picture. The development of machine learning algorithms has improved the accuracy and dependability of applications for object recognition and picture categorization. Therefore, machine-learning methods are utilized across diverse fields. Specifically, deep learning approaches find use in several medical domains, such as automated segmentation of the left ventricle in cardiac MRIs, retinal fundus pictures for diabetic retinopathy diagnosis, and chest X-rays for abnormality identification and localization[10,11]. Hence, it is valuable to explore deep learning-based methods for the identification and counting of blood cells in smear pictures

All living organisms are made up of fundamental biological units called cells, which come in a variety of sizes, shapes, and functions. The process of identifying certain cells or structures within an image is called cell image segmentation. It is an important step in the procedures of biomedical image analysis, where the pixels in the picture are segmented into regions of interest (ROI), as Figure 1 illustrates. This method is essential for many areas of study, such as drug discovery and the characterization of cellular dynamics in both healthy and pathological situations [12]. The ability to accurately portray cells and subcellular structures has been made possible by recent developments in high-resolution fluorescence microscopy.

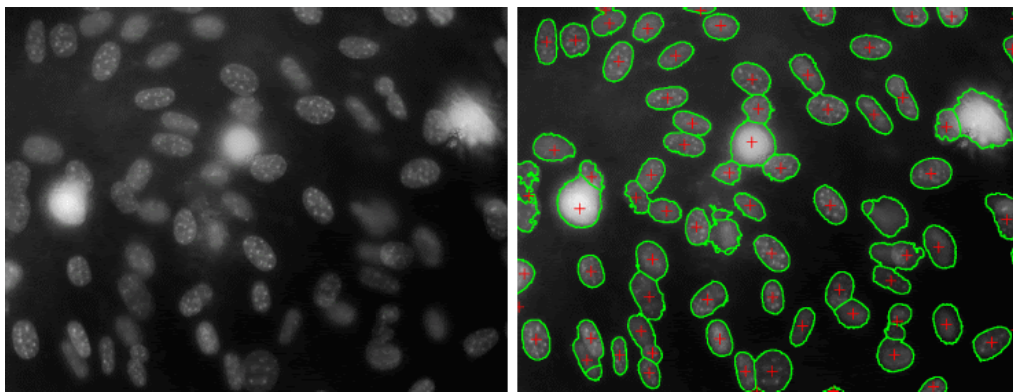


FIGURE 1: The result of segmented cells (right) and an image captured using fluorescence microscopy (left). Green curves and red plusses, respectively, are used to depict the borders of the discovered cells and the ground truth cell centroids [12].

2. Literature Survey:

The two primary methods used in the automatic blood cell calculating procedure are machine learning and image processing[13,14,15]. Red blood cell (RBC) counting was made possible by the introduction of an image processing method by Acharya and Kumar [10,]. They looked at blood smear images in order to count red blood cells and differentiate between abnormal and normal cells. Granulometric analysis was employed by the researchers to differentiate white blood cells (WBCs) from red blood cells (RBCs). They used the labelling method in conjunction with the circle Hough transforms (CHT) to quantify the cells after extracting WBCs using the K-medoids technique. Sarrafzadeh et al. were the first to quantify RBCs in grayscale pictures using the circular Hough transform. Kaur et al.'s suggestion[16] was to automatically count platelets from images of tiny blood cells by using the size and shapes of the platelets recognised by the circular Hough transform (CHT). Cruz et al. [17] presented an image processing method for counting blood cells that counts blood cells by identifying them using colour, saturation, value thresholding, and linked component labelling. Red blood cells (RBCs) may be counted semi-automatically by utilising a Hough transform to distinguish between their oval and biconcave morphologies, as suggested by Acharjee et al. [18]. A technique for automatically counting red blood cells (RBCs) utilising support vector machines (SVM) and spectral angle imaging was described by Lou et al.

Zhao et al presented a CNN-based automated method for WBC identification and categorization. After first categorising WBCs using microscopic images, they used CNN to categorise different kinds of WBCs. A technique for categorising WBCs into five distinct groups using three classifiers—two SVMs and one CNN classifier—was reported by Habibzadeh et al. [19]. They also used trained CNNs, ResNet, and Inception Net to count WBCs from segmentation images, and they used colour space analysis for segmentation. After applying patch size normalising to previously processed images, Xu et al. employed CNN to categorise RBC shapes from microscope photographs of sickle cell anaemia patients [20,21, 22]. This study covers a wide variety of significant aspects of the cell counting process, including picture kinds, methodologies used, cell types, and performance, as Table 3 illustrates.

Table 3: Examination of Different Cell Counting Methods in Literature

Sr.No.	Image types and dataset name	Method used	Cell output types	Performance Parameters	Restrictions and Potential Area
1[2024]	Normal and —Abnormal microscopic images. The number of images is 85 samples for each category (with a total of 170 images). The types of images are RGB images with (.jpg) and (.bmp) extensions	Deep Learning method	leukemia in blood microscopic images	Confusion matrix TPR(True Positive Rate) TNR(Ture Negative Rate) PPV(Positive Predictive Value) NPV(Negative Predictive value)	Other features could be extracted in the future, and all of them can be used in machine learning classification processes like Support Vector Machine (SVM) or deep learning methods like neural networks (NNs) to build a model and classify huge number of blood microscopic images into normal or images with leukemia automatically.
2[2023]	Synthetic training data set of microscopic images	3D Nuclei Segmentation (NISNet3D)	Red Blood cell White blood cell	Aggregated Jaccard Index Number of True Positive detections number of False Positives number of False negative Mean Precision Mean Precision Average	In the future, it will explore the generation of synthetic nuclei segmentation masks to better simulate complex nuclei morphologies and more realistic densities and distributions. Further extending the current SpCycleGAN to generate fully 3D

					synthetic volumes to include a model of a microscope point spread function
3 [2022]	Cells of bacteria (256 pictures)	Chip-based image cytometer that counts E directly by using a low-volume, thin-gap counting chamber consumable.	Bacterial tissue	Aggregate Viability, size, concentrations of living and dead cells, total cell	Can be used for large-scale applications on actual datasets
4 [2022]	Glass slide microscopic pictures	Using a Fossomatic FC Cell for Cytometry	Blood smear or blood cells	computed outcome in flow cytometry. tiny platform for smartphones that portable	minimal precision while using different cell types.
5. [2022]	Images from microscopy	Density Regression-oriented Approach	blood molecule	MRE and MAE. Average Absolute Error Cellrage Relative Error	For real-time clinical applications, real-time datasets must be employed in this study rather than synthetic ones. They can help with a number of issues, including item counting in congested situations
					that involves more than just cells.
6. [2021]	150 blood smear slides of whiSmear of bloodlls	Hemocytometer Hybrid (Random forest plus fuzzy logic)	Bloodsmear Cell	The system's sensitivity and specificity are determined to be 79.6% and 96.4%, respectively.,	By incorporating GPS into the building of a comprehensive illness map that is broken down by both region and year, the efficacy of this approach may be further enhanced.
7.[2021]	photos taken by a camera (blood cell pictures)	Hemocytometer using the ImageJ programme	Blood Red blood cell	Compared to hand counting, this method is around ten times quicker and produces more accurate and consistent results.	

8.[2021]	microscopic pictures of blood.	Techniques for automatically selecting thresholds	Red blood cell	The quantity of red blood cells, precisely and reliably	High-quality picture databases should be used to test it, and vice versa for poor performance. More comparisons are necessary to provide equitable performance comparisons.
9.[2021]	photos of microscopic cells	Papicture the K-means clustering algorithm	tiny picture of a blood cell	Quickly and accurately determine the size, type, and number of cells.	It lacks the ability to recognise and identify distinct cells in the same picture.
10. [2020]	Histological pictures	Convolutional Neural Network	Cell image data	Total mean error departures from the norm	This study is applicable to real-time datasets.
11[2020]	three-dimensional cell membrane	Workflow for 3D cell segmentation based on deep learning, 3DCellSeg	Plant (ATAS), Animal (HMS), Plant (LRP), and plant ovules	cell membrane identification of cancer cells	An end-to-end deep learning pipeline may be created to directly segment cell instances in 3D pictures.
12[2019]	The pee of individuals with bladder cancer and urinary tract infections	The Otsu algorithm	Image of a urine cell	Four parameters (urine cell, dice, jaccard, accuracy as well as recall	There is a dual mode for this work. involves image processing and recognition, as well as droplet microfluidics.
13[2018]	Images of Human Cancer Cell Lines under a Microscope	Transfer Learning and Deep Learning CNN	tiny picture	<i>equivalent to mean absolute error</i>	The accuracy of this method's calculations of cell morphological parameters is lower.
14.[2018]	Picture blood molecule cells	Hough Conversion	Blood cell	Effective and economical computer vision automated red blood cell counting system	Real-time microscopic picture implementation of this work is possible. <i>taken with a microscope linked to a digital camera.</i>
15.[2018]	Pictures of blood samples	Segmentation techniques	pictures of the bone marrow	Accurately dividing closely packed leukocytes in bone marrow pictures.	Only in bone marrow cells is there segmentation
16[2018]	microscopic pictures	Bradley's technique, depending on region	The NIH3T3 dataset: A group of forty-nine pictures	Nuclei cells automatically recognise and divide contact cells.	More comparison with alternative automatic cell counting techniques is required.

17.[2018]	photos of microscopic cells DATA SYNTHETIC	neural system	NSCs, or neural stem cells picture dataset made up of 232 movies from 8 trials and 1,130,496 monitoring connections in total	Monitor Living Cells	This technique is applicable to both 2D and 3D photos.
18.[2016]	Images of microImages of cells and dataset of image cytometry	The Cellsegm Method	Cell images	Accurately dividing cells	Comparison with alternative techsegmentation of cellsy.
19[2015]	Pictures of blood cells	Deep learning methodologies pictures of cellse blood cells	95.45% accuracy in WBC identification and 90.49% accuracy in WBC distinct categorization.		
20.[2015]	pictures of cancer cells/MRI pictures of cancer cells	Kohonen's algorithms for competitive learning (KCL)	cancerous cells	Cell segmentation The metastatic cell	reduced precision with different cell types
21[2014]	cells images	Cell identification using deep learningnR-CNNNN	cancerous cellsThis approach yields a median error of about 1% of the total number of cell units.itFrom 71% to 85%, the accuracy rate on average rose.85%.To count and segment cells, it is necessary to compare them with other types		
			of cell		
22.[2013]	Red Blood cells	Segmentation Technique	blood cells	The suggested approach has been put to the test in a number of studies, and the results show that, up to a particular flow rate, cells may be effectively counted and accurately identified using image processing techniques.	In a later project, the camera's serial interface is used to directly capture photos. and completed online. This can reduce the overall computation time as the duration of file reading.

23.[2013]	Comparing microscopic pictures with cells	Method of segmentation ANN		The experimental findings demonstrated that the suggested method's average MIS, INC, and ACC values are only 3.31%, 3.49%, and 96.69%, respectively.	
24[2007]	Histological images of the digestive system and lungs.	Method of classification	The picture was obtained with a 60x magnification and 256 grey levels under immersion, beneath a microscope	Classification of the picture automatically (cancerous or not) is conducted at last.	Classification limitations resulting from the use of several photos of the same tissue
25.[2005]	Artificial materials/Supported Image Interpretation	Synthetic Neural Network	NIH Picture	At a 100x magnification, the correlation index was almost identical to individual variability.	may produce positive results when used with a real-time dataset.
26 [2002]	pictures of bone marrow specimens that included 2,433 Leukocytes in clusters	CNN Neural Convolution Network		The formula effectively segments tightly grouped data leukocytes in pictures of bone marrow	
27.[1999]	Images of microscopic cells	profound understanding	erythroid cells in the bone marrow	87.16% precision, 87.45% recall, 86.33% accuracy, and 87.30% F1 score. ErythroidCounter achieved 90.6% accuracy, 90.2% recall, and 90.8% F1 score in the effective identification of erythroid cells.	This model has more Ideal for classifying images of erythroid cells and enhancing the precision of cell categorization.
28[1998]	photos of microscopic cells ..	Neural Inflammatory Network	Histological pictures	Cell division	Additional research involving Automating the selection of cells
					and trimming There is a requirement to increase the number of classifications. kinds (that is, cell types), as well as a misclassification analysis

29 [1996]	Images of blood cells	GVF representation Vector Flow Gradient	An artificial collection of microscopic pictures	Fibroblasts, amoeba, and leukocytes or malignant cells	Only the cells that were manually initialised are tracked.· incapable of managing items later in the action, joining the scene.increased resilience. it may be used to a greater variety of picture modalities and to image noise.
30[1986]	Pictures of blood cells	Transformation of euler's coordinates and histogram	Pictures of blood	cells from the bone marrow	This task might be completed automatically.
31[1985]	Images of microscopic cells and liver tissue	automated cutoffbased (computer-based) analytical techniques	Set of data including 25 slides	Hepatocytes,leukaemi a and sinusoidal	Outcomesof liver tissue component image segmentation, including hepatocytes,leukae mia, sinusoidal
32[1977]	Microbiological cells/Plastics	Algorithms for thresholding	photos of microscopic cells	Analysis of cell morphology and counting.	The Bensen technique can be further optimised by changing the threshold selection procedure.

3 Cell Counting Process:

A tiny cell picture is initially acquired and used as an input before cell counting. Next, the image is pre-processed by removing unnecessary objects, modifying the brightness and colour contrast, and segmenting the cells[19]. The following step, called cell detection, compares basic cell characteristics to ascertain if a sample is a cell. The next step is counting to see if the object is identified as a cell [23,24]. At this phase, the cells in the given image are counted using a range of techniques. The presence of the cell object is next verified by examining the location of the discovered cell. Figure 2 depicts the structural arrangement of the cell counting procedure.

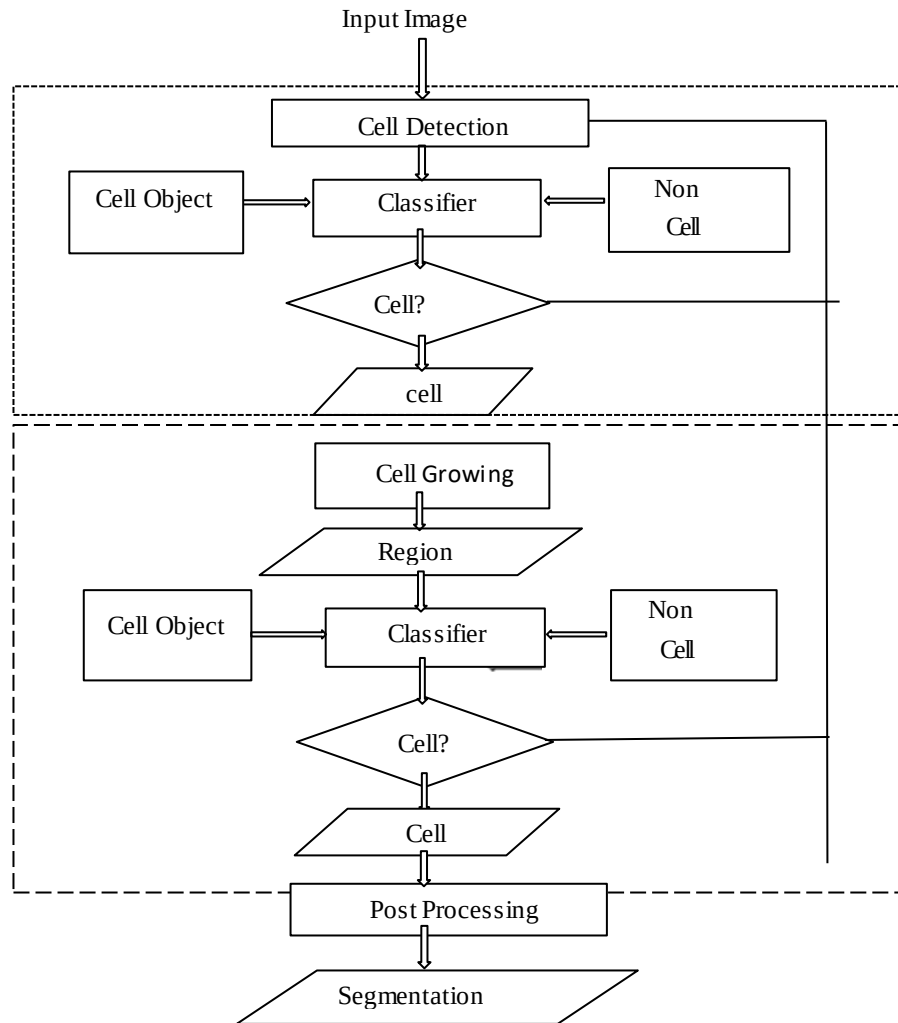


FIGURE 2: Cell counting system's functional organisation, with cell development, detection, and post-processing separated to prevent false positives [24]

Stage 1: Cell Detection

Accurate cell detection is difficult since there is a chance of both over- and under-detection. Every cell consists of a nucleus that is originally viewed as a single unit and is surrounded by cytoplasm[25,26]. Problems with cytoplasm segmentation include backdrops, different hues, and overlapping cells. Because there is little overlap, segmenting the nucleus is comparatively simpler. It is possible that Computerised Image Segmentation (CIS) handles under- and over-detection more skillfully.

Here are some crucial details about cell detection:

1. While an elliptical representation is more accurate, a circular form can suffice.
2. Nuclei are commonly used as model cells.
3. Each item of interest should have at least one local intensity maximum.

Stage 2: Identification of Cell Regions

The cell region of interest is created when cell detection is complete. Region growth or border deformation can be used to carry out this procedure, which frequently makes use of machine learning techniques[27,28]. When used for boundary deformation, active contour models create precise curves around cells under the influence of both internal and external variables.

Here are some essential concepts for region expansion:

1. Use edge points to find the distance to the nearest edge.
2. Modify the code to incorporate gradient direction.
3. Utilise an intermediate value to trace concavities in nuclear and cell boundaries while smoothing bright areas.

Stage 3: Post-processing

Oversegmentation and undersegmentation are frequent errors in cell picture segmentation. The oversegmentation can be corrected by size and form criteria. Manual evaluation, for example, can determine the normal shapes or sizes of cells[29,30]. If a single cell is split into more than two regions, a region merging technique can combine excessively separated cell portions back into one.

Here are some crucial details about post-processing:

1. Tiny cells disintegrate more easily due to their size.
2. Generally, the image's noise level is below the threshold.

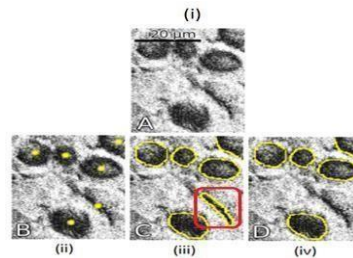


FIGURE 3: The top original picture (i) Cell development (ii) Cell detection (iii) After processing to confirm the over-segmentation (false positive red border)[31]

3.1 Using Python to Implement Cell Counting

1. Pandas and further imports Before importing any Python libraries, import pd Numpy so that np may be used for data manipulation. While using arrays, Sklearn: To get ready, Shutil, Zipfile, OS, Sys, random, Glob, matching data, cv2, displaying the picture, and Matplotlib #data visualisation may all be used to conduct file operations. In the following phase, the data extraction from the downloaded dataset is finished. The dataset (annotated data) was then loaded. In this step, we read the annotations in the dataset file. csv file, as seen in table 4 below, which calculates the width, height, and middle of the picture.

Table 4 Calculated the width, height, and middle of the picture

S.No	Image	Xmin	Ymin	xmax	ymax	label
0	image-100.png	0.000000	0.000000	25.190198	40.816803	Rbc
1	image-100.png	15.010502	0.000000	68.337223	23.527421	Rbc
2	image-100.png	25.017503	16.021004	78.374562	73.735123	Rbc
3	image-100.png	75.565928	1.061844	140.248541	45.591599	Rbc
4	image-100.png	77.483081	23.290548	131.936989	74.806301	Rbc

Following that, a label has been put to the cells that were detected, displaying an example image in FIGURE 4 along with its ground truth and an iterative process for various items. Next, as seen in FIGURE 5, apply distinct colours to various item classes and add bounding boxes to the picture.

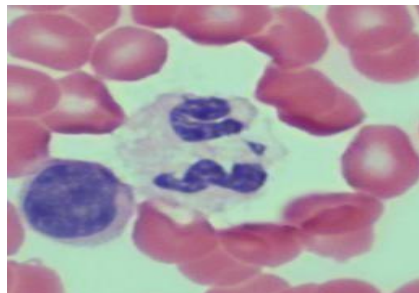


FIGURE 4: Input image

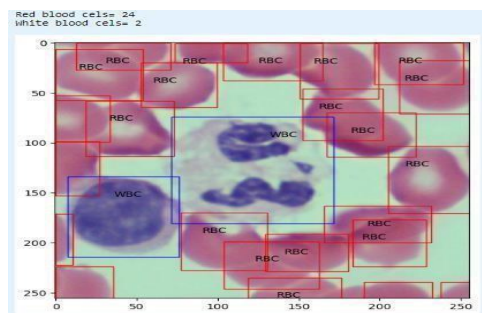


FIGURE 5: Output (Red box- red blood cell), Blue boxes(white blood cell)

4. Difficulties with Cell Counting:

The development of a well-tuned system and enhanced outcomes require a strong grasp of technology, cell structure, and algorithms. There are a few challenges to overcome, which are discussed in the sections that follow. The extensive interconnectivity of our body's cells makes them challenging to detect and research, as Figure 6 illustrates.

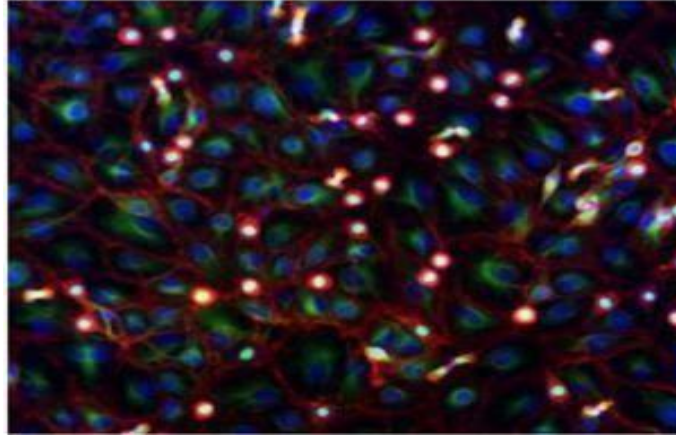


Figure 6: Difficulty in Joining Discontinuous Object Borders That Must Be Connected

a) Semantic Gap: Manually separating a cell from its background requires a great deal of deep understanding and heuristic techniques, which can be difficult to do. Automated segmentation needs to be algorithmic, albeit [32]. It has been observed that some image formats are not well suited for automated segmentation algorithms, which may cause the images' original attributes to be lost in the process. Error probabilities and algorithmic implementation problems also occur.

b) Variable Physiognomy: These abnormalities are caused by extensive structural alterations as well as unwanted noise in the form of cellular detritus and biomaterials [33,34]. There are numerous types of cells that are made of detritus derived from other sources. Significant changes in intensity levels can result from variations in a cell's brightness and flaws. This poses difficulties for segmentation that is automated. When artificial materials or trash are mistaken for cell sections, automatic segmentation can also result in misconceptions and identification problems.

c) Irregular Cellular Corona: This problem may arise from an image with excessive blur. Cell images show variable highlight effects and shadows from different perspectives, as well as varying picture contrast levels and radiosopic power [35]. Automatic segmentation is prone to mistakes because of this miscommunication between the backdrop and the cells.

d) Cell Adhesion: A cell image's many tightly knit cells make it difficult to depict each one independently, which is essentially needed for cell picture segmentation. As a result, cell picture segmentation automatically gets challenging. Cell segmentation is further hampered by the concentration of cells in the cytoplasmic outer layer that vary in size and shape [36]. It has been determined that hybrid cell image segmentation is necessary to solve these problems and create new systems.

5. Cell Counting Techniques

Image segmentation uses four primary approaches (histogram-based, boundary-based, region-based, and pixel-based) to identify, analyse, and assess cells and their subareas[37,38]. It has been established that a variety of perceptual techniques based on categorization and grouping are useful in the detection of cancer. To improve cell images and get the best results, pre-processing will be done using morphological and image filtering methods.

5. Cell Counting Techniques

To detect, analyze, and evaluate cells and their subareas, image segmentation employs four basic methodologies: histogram-based, boundary-based, region-based, and pixel-based. Numerous perceptual methods based on grouping and classifications have been demonstrated in the process of cancer diagnosis. Pre-processing will be carried out utilizing morphological and image filtering techniques to enhance cell pictures and achieve optimal results.

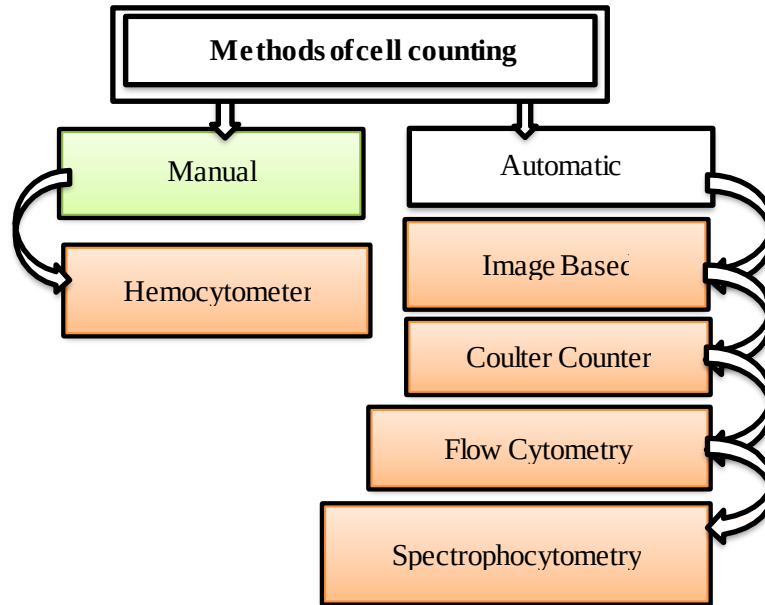


Figure 7: Cell Counting Algorithms

a) Hemocytometers: A fundamental instrument for precise cell measurement is the hemocytometer. With further divisions, their gridded chamber design allows for exact cell counts. Reliability is ensured by cleaning with 70% ethanol and lens paper before to use[39]. The use of trypan blue makes it easier to detect cell viability, which is essential for accurate findings. Because of their accuracy and simplicity, hemocytometers are still widely used in cell counts. Strict cleaning guidelines must be followed by users to ensure peak performance. Experimental integrity depends on improved data quality, which is achieved by viability evaluation[34]. Hemocytometers, in general, provide a reliable answer for simple cell counting requirements.

b) Coulter Counters: By measuring electrical resistance across microchannels, Coulter counters revolutionised the counting of cells. The counting procedure is streamlined by automated operation, which increases productivity. For proper analysis, homogeneous dispersion of the cell solutions is ensured by careful mixing and dilution[40,41]. Automated cell counters and Coulter counters function similarly, which emphasises how dependable they are. Their efficacy stems from their ability to accurately quantify cells, which is essential for a range of scientific uses. Coulter counters are sophisticated devices, but for best performance, they need to be set up and maintained correctly. They serve as a pillar of contemporary cell counting technologies. Their versatility in managing various cell kinds and dimensions renders them indispensable for biological investigations.

c) Flow Cytometers: b) Flow Cytometers: Using fluorescence detecting technology, flow cytometers are at the forefront of cellular analysis. Precise counting is facilitated by their ability to distinguish between various cell types based on protein expression[42]. Despite the need for intricate testing and antibody incubations, flow cytometers are easy to operate. Their adaptability includes detailed cellular investigation in addition to cell counts. Flow cytometers are incredibly complex devices, yet they provide dependable, repeatable findings that enable researchers all around the world. Their usefulness in a variety of sectors is highlighted by their capacity to identify subtle variations in cell

populations. When it comes to comprehending cellular dynamics and disease processes, flow cytometry is still invaluable.

d) Spectrophotometry: d) Spectrophotometry: Using light absorbance, spectrophotometry provides a non-invasive way to measure cell density. Accuracy is improved by comparison with known densities, even if turbidity may influence measurements [43-47]. A simple method for determining relative cell density is to use a spectrophotometer. Even with its drawbacks—like possible outside interference—spectrophotometry is still a useful instrument. Its use is not limited to cell counting; it may also be applied to different biochemical tests. To reduce mistakes, users should evaluate absorbance measurements carefully. A key tool for measuring biological components in research and diagnosis is spectrophotometry. Because of its ease of use and adaptability, biological laboratories all over the world rely on it.

6. Future Extent and Paths for Research

Future studies in cell counting might help to solve current problems and move the field closer to automated solutions that are more precise and dependable. To increase segmentation robustness and accuracy, one possible approach is the creation of hybrid approaches that combine machine-learning algorithms with conventional image processing methods like watershed and snake algorithms. Furthermore, even with intricate and diverse picture datasets, more research and development of deep learning techniques, in particular convolutional neural networks (CNNs), [48-51] can improve the recognition and categorization of various cell types. Furthermore, as segmentation accuracy can be greatly impacted by uneven cell corona, varied physiognomy, and cell adhesion, future study can concentrate on improving cell counting methodologies to address these particular issues. Research may be conducted on sophisticated image-preprocessing techniques, such as morphological and filtering procedures, to enhance the quality of cell images and enable more accurate counting[52]. In addition, the creation of benchmark datasets and standardised assessment criteria is required in order to impartially evaluate the effectiveness of various cell counting methods and algorithms[53]. This would encourage innovation in the sector and make it possible for researchers to compare results more efficiently.

In summary, there is a lot of potential for improving automated cell counting systems through the combination of deep learning, machine learning, and sophisticated image processing approaches. Future research can help produce more dependable, accurate, and efficient cell counting methods with a wide range of applications in biological research, clinical practice, and medical diagnostics by tackling existing issues and investigating novel approaches.

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Funding Information- Not Applicable

Author contribution-

1. This survey paper explores numerous algorithms pertaining to automatic cell counting techniques in machine learning and deep learning for medical image analysis.
2. The paper offers a comprehensive review of two decades-long research in the field of cell Counting.
3. The reviews have been conducted formally, encompassing the most recent advancements in microscopic image cell counting techniques. Unlike other surveys, real-time applications are discussed along with the significance of cell counting in microscopic images.

Data Availability Statement- Dataset is available on <https://github.com/MahmudulAlam/Complete-Blood-Cell-Count-Dataset>

Informed Consent. NA

Authors Biography:



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