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A Systematic Survey on Biological Cell Image Segmentation and Cell Counting Techniques in Microscopic Images Using Machine Learning

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Abstract
The article focuses on the concepts of Cell Image Segmentation (CIS) and the gradual introduction of cell counting. Motivated by the rapid development of Machine learning (ML) methods, which is carried out in this investigation. ML is evolving from theory to practical applications, with deep neural network models extensively used in academia and business for various applications, including image counting and natural language processing. These advancements can greatly influence medical imaging technologies, data processing, diagnostics, and healthcare in general. Main objectives of the research are to provide an overview of biological cell counting methods in microscopic images and to explore deep learning (DL)-based image segmentation approaches. The study expertly describes current trends, cutting-edge learning technologies, and platforms utilized for DL approaches. Cell counting is one of the most researched and challenging subjects in computer vision systems. Academics are increasingly interested in this area due to its real-time applications in biology, biochemistry, medical diagnostics, computer vision-based cell tracking systems for large populations, and stem cell manufacturing. Counting cells in the biological field is beneficial. For instance, the ratio of white blood cells to cancer cells in the blood can help determine the origin of a disease. Biologists also need to count cells within cell cultures to monitor the time-dependent growth of cells during bacterial experiments. Numerous methods for cell counting have been developed, after addressing the challenges with Cell Counting algorithms; the article explores promising future directions in CIS and cell counting research fields.

Keywords Cell Image Segmentation · White Blood Cell · Biomedical · Biology · Automatic Cell Counting · Image Analysis

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1 Introduction of Cell Counting and Motivation

Cell counting, also known as cell enumeration, is the process of determining the total number of cells, including red blood cells and white blood cells, in human blood. It is a fundamental measure used in biotechnology for various purposes, such as advanced therapy, medical diagnostics, and bio-manufacturing [1]. Cell number is essential for determining the correct dosage for cell-based treatments. Cell counting plays a vital role in various fields of research, including biological applications, genetics, biochemistry, and medical diagnostics and treatments. Additionally, cell counts are essential for preparing cells for research, assessing cell viability, and determining growth rates. Cell counts must be accurate, trustworthy, and rapid to measure cellular responses. For example, the number of leukocytes, known as white blood cells, in the blood indicates the presence of a disease during a medical diagnosis. The most prevalent type of leukocyte, neutrophils, defends the body from bacterial infections. Similarly, lymphocytes, another type of white blood cell, defend the body against viral diseases. Another example is the reappearance of cancer following treatment of cancer cells. To prevent the issue from recurring, the system can automatically monitor and preserve both normal and abnormal cells related to the illness [2]. Researchers have developed both manual and automated cell-counting techniques. Although manual methods are labor-intensive and error-prone, they are often preferred due to their cost-effectiveness compared to other methods. Automated cell counting techniques, such as flow cytometry, have been developed to provide faster and more accurate results. In flow cytometry, cells are individually passed through a narrow channel, which is a key feature of this technique. One of the active cell treatment drug tests in Great Britain is different from conventional pharmaceutical production patterns, although it shares similarities with them. The efficient and practical advancement of mitochondria to large items, the efficient differences and cleansing of goal cellular processes, as well as the price and Good Manufacturing Practice (GMP) interoperability of existing processing technologies, are significant barriers to the adaptation of stem cell transplantation methods utilizing progenitor cells (PSCs) [3]. Nevertheless, if these limitations can be overcome, autologous medicines would have less diversity in growth in the developing world and would be made on a greater scale, enabling them to more effectively treat conditions with a higher prevalence. Nilsson et al. state that white blood cells (WBCs), also referred to as leukocytes, play a crucial role in human innate immunity. In order to battle infections, these cells destroy bacteria, viruses, and other unwanted diseases. An abnormally high white blood cell count may indicate the presence of a rare illness. Typically, diseases that harm epithelial cells can compromise the body's ability to eliminate or control infections. Therefore, medical microbiology and malignancies focus on white blood cell counts due to their diagnostic significance. A total WBC count may identify diseases concealed inside the organism and notify clinicians of undetected problems such as autoimmune disease, immunological deficits, brain disorders, and malignancy [4]. A WBC count may suggest the existence of an illness affecting white blood cells, but it can also help understand the underlying causes. A blood smear examination is a technique used to examine blood cells under a microscope. These techniques make it possible to automatically count cells and determine things like cell size. When different cell types are examined side by side, the ability to classify cells based on their size allows researchers to determine how many cells are present in each group. For instance, counting the different types of white blood cells can be used to identify the disease type. One of the flow cytometry methods, electrical impedance spectroscopy, examines the impact of cell migration on the electromagnetic field applied to the microchannel,

allowing the detection, counting, and physical characteristics of cells. Yet, the necessary equipment for this method is costly. Wang et al. developed a framework to prevent double counting and enhance the accuracy of counting [5–7]. To test how well the method works in different scenarios, an evaluation has been conducted using new images. The technology for detecting and counting individual cells using visual methods is displayed in Fig. 1. Figure 1 displays images of blood cell microscopic images as the dataset. Subsequently, the cell detection algorithm was applied to the dataset. To detect the cells, the model has been trained on key features such as shape, size, and color. Next, the detected cells are counted to determine the total number of cells [8, 9]. The survey paper explores numerous algorithms pertaining to automatic cell counting techniques in machine learning and deep learning for medical image analysis. The paper offers a comprehensive review of two decades-long research in the field of cell segmentation.

1.1 Key Challenges and Research Gaps

Some of the important key challenges and research gaps have been identified, which can be addressed while developing cell counting techniques and applications for medical image analysis in a clinical setting. Based on the literature review, some research gaps related to cell counting have been identified:

1) **Time-consuming process** [10]**:** Automated cell counting is as fast as it sounds. You prepare your slide, insert it into the appropriate slot, choose your cell counting protocol, and press the count button. Wait a few seconds, and the results will be presented in a document.
2) **Delicate procedure of coverslip preparation** [11]**:** Let's take a look at the most common method for manually counting cells, the hemocytometer. First, you must prepare a plastic or glass hemocytometer coverslip by rinsing it with rubbing alcohol. Still unfinished, you must attach it to the hemocytometer. A delicate procedure is prone to mistakes. With automated cell counting, you remove a chip from its packaging, load a suspension of cells onto it, and insert it into the automated cell counter slot.
3) **Different staining** [12]**:** Specimens can make it challenging to distinguish between a cell's nucleus and cytoplasm, especially for techniques that divide according to color. Cell images have been produced using a variety of techniques.
4) **Morphological diversity**: Cells appear in a variety of shapes, textures, and orientations in microscope photographs. Blood smear images' ability to be segmented accurately is hampered by the morphological diversity of the cells and the murky, complicated background.

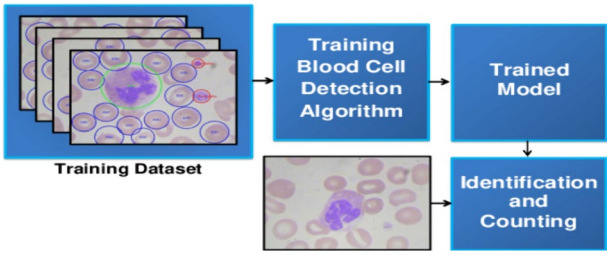

Fig. 1 Training dataset cell count (Source: IET Research Hub)

5) **Irregular borders between cells and nucleus** [13], which can vary greatly within cells, lobed nuclei. Due to overlapping and distorted cells, it could be difficult to track changes in cell boundary topology. Problems could arise when cells and their nuclei combine or split, or when boundaries are concave, convex, or have sharp edges.
6) **Number of cell count** [14]: For segmentation, it is crucial to count the number of cells in each area. Unlike the watershed transform, pixel replication does not estimate the number of cells in each area.
7) **Lack of generalizability** [15]: Many researchers ignore the issue of the network response's generalizability in the event that the data source changes. That is what a transformation in a data acquisition device will do to image features, like illumination or color intensity levels, for example.
8) **Lack of sufficiently large imaging datasets** [16]: The requirement for very large imaging datasets presents another issue with Deep Learning Classifier (DLC) based networks. As a result, there will be significant storage and memory needs, and the networks will need a lot of training time.
9) **Microscope illumination**[17]**:** The nucleus and cytoplasm may have varied color distributions due to differing microscope illumination techniques and uneven lighting, making it difficult to distinguish between these areas.
10) **Manual errors counting the cells**: Relying on human vision and finger clicks on a clicker will result in inaccurate counts. Why not rely on the automated cell counter, which uses a sophisticated detection camera and algorithm to produce consistent and accurate results? Each time the cell counter is used to count cells, the system will respond consistently.
11) **Cell count variability:** An automated cell counter can accurately count a variety of cell densities. If too few or too many cells populate the field of view, the count may not accurately represent the stock solution.
12) **Challenging differentiation between cells and debris**[18]**:** For instance, Luna cell counters can distinguish between cells and debris much more easily than the human eye. The Luna-FL, which utilizes two fluorescent colors for detection, is ideal for counting primary and stem cells, which typically contain a great deal of debris.
13) **No standardized protocol:** For example, Luna cell counters can differentiate between cells and debris far more easily than the human eye. Utilizing two fluorescent colors for detection, the Luna-FL is ideal for counting primary and stem cells, which typically contain a great deal of debris.

Several issues need resolution, with the major ones described here. Due to the close proximity of our body's cells, as seen in Fig 2, detecting and analyzing them when they are connected is challenging. To enhance outcomes and establish a well-controlled system, we must address these challenges with a comprehensive understanding of technology, cell structure, and algorithms.

1.2 Terms Abbreviated Throughout the Paper

Table 1 list of abbreviations utilized in this paper, particularly in the medical field.

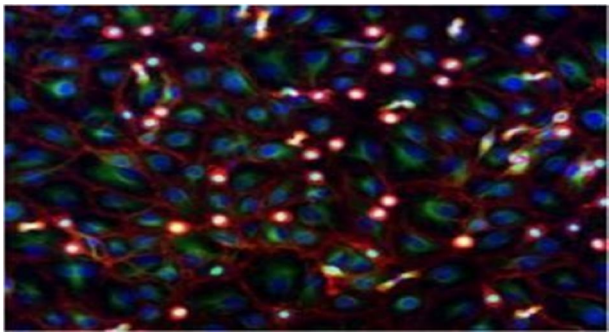

Fig. 2 The problem of connecting discontinuous object borders that are nonetheless need to be connected persists

Table 1 List of abbreviations

S.no	ML	Machine learning
1	US	Ultrasound
2	NLP	Natural language processing
3	CNN	Convolutional neural network
4	DL	Deep learning
5	MRI	Magnetic resonance imaging
6	ANN	Artificial neural networks
7	PET	Positron emission tomography
8	CT	Computed tomography
9	CIS	Cell image segmentation
10	EBCT	Electron beam computed tomography
11	fMRI	Functional mri
12	MRS:	MR spectroscopy
13	SPECT	Single photon emission ct
14	DLC	Deep learning classifier
15	ROI	Region of interest
16	RBC	Red blood cell
17	WBC	White blood cell

2 Contributions of Research

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 segmentation.
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.

3 Research Methodology

The objective of the narrative review is to identify research that developed or validated cell-counting models to detect any disease features from histopathology and medical imaging data. As depicted in Fig. 3, the initial research criteria focused on the classification of various biological cell images under "Cell image segmentation," while the secondary search criteria centered around "cell detection and counting" within the domain of "Computer vision." This article focuses on various cell counting and cell segmentation techniques from microscopic images. Out of 532 publications, 96 were carefully selected based on their relevance to the study. Important methods and the criteria for the study's selection were included in the survey.

Research papers in this field have been classified into three categories:

1. **Primary Keywords:** This category involves searches based on primary keywords related to the field of cell counting, such as biomedical images, cell-counting, types of cells, and images.
2. **Additional Keywords:** Here, additional keywords were added to the primary keywords, such as cell counting from microscopic images and image segmentation.
3. **Secondary Keywords:** This section involves searches based on secondary keywords, like techniques for cell counting and the application of cell counting.

3.1 Research Evaluation Standards

Figure 4 illustrates the selection criteria utilized in this study. In the first phase, a keyword search has been conducted to eliminate redundant and irrelevant materials from the selection process, resulting in 532 articles being identified. Subsequently, in the second phase, a title-based search has been performed, focusing on automatic cell counting techniques, leading to the selection of 190 papers. Following this, a thorough evaluation

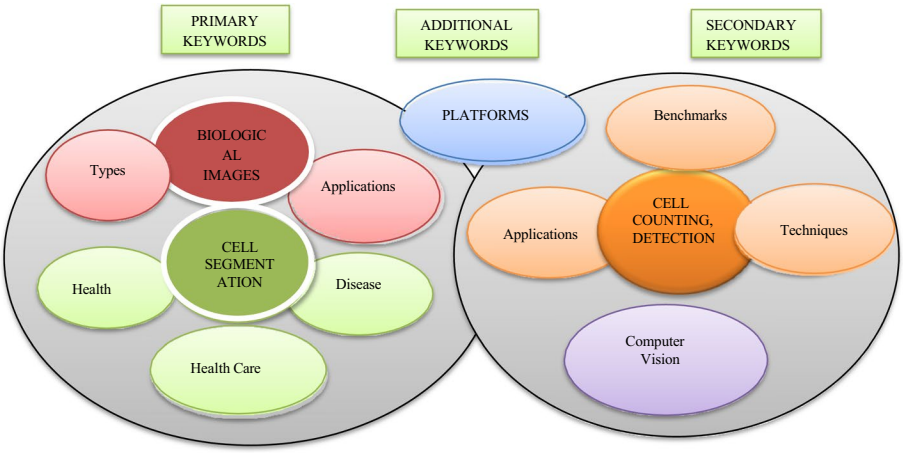

Fig. 3 Primary, secondary, and supplemental keywords are used to choose the articles

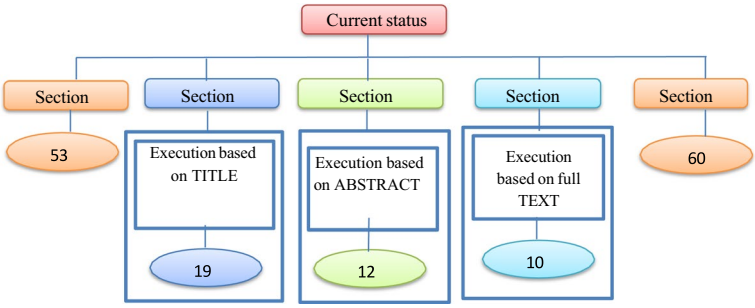

Fig. 4 Study selection process

of the full text was undertaken, resulting in the selection of 101 research manuscripts. Finally, from these 101 papers, 60 research articles have chosen for inclusion in the current study.

3.2 Research Questions

The strategy of incorporating research questions into the study aligns with the review technique. These inquiries are crafted to encompass every aspect of the proposed paper, including the utilization of deep learning and machine learning in healthcare analysis, medical image processing, and other critical areas essential for delineating the study process. Table 2 delineates the rationale behind the research questions (Fig. 5 highlighting the differences between traditional and deep learning approaches for cell counting." The latest method is based on images).

3.3 Information Sources

Table 3 depicts the extensive databases that are employed in this review process as a search platform.

Table 2 Research questions utilized in the review process

Sr. No	Review questions
Q1	What is the duration of this survey?
Q2	What are the current topics for research in biological cell counting in microscopic images?
Q3	What are the challenges when developing applications for medical cell image analysis
Q4	What is current research in cell images?
Q5	What are the various automatic cell-counting techniques?
Q6	How soft-computing and machine learning helps to develop cell-Counting technique?
Q7	What are the different cell counting based real-world applications?

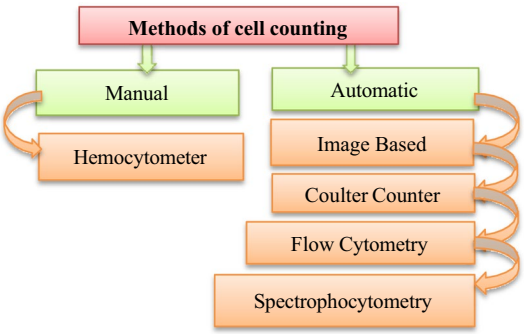

Fig. 5 Cell counting methods

Table 3 List of information sources

S.no	Platform
1	Web of science (https://www.webofknowledge.com)
2	Science direct (https://www.sciencedirect.com)
3	Springer(https://www.springerlink.com)
4	IEEE explore (www.ieeexplore.ieee.org)
5	Elsevier (www.elsevier.com)
6	Google scholar (www.scholar.google.co.in)

4 Cell Counting: A New Age

4.1 What is Cell Counting?

Cell counting is a vital technique used in scientific research and medical diagnostics to quantify the number of cells in a sample accurately. This process involves determining the concentration of cells within a specified volume of the sample. It plays a crucial role in assessing cell proliferation, viability, and response to treatments in laboratory studies. In clinical settings, cell- counting aids in diagnosing diseases, monitoring patient health, and evaluating treatment outcomes. Biotechnological and pharmaceutical industries rely on cell counting to monitor cell cultures during the production of therapeutic products [10]. Various methods, such as manual counting under a microscope, automated cell counters, and flow cytometry, are employed for cell counting, each offering different levels of precision and throughput. Accurate cell counting is essential for understanding cellular behavior, disease progression, and the development of new treatments and therapies (Fig. 6 shows different types of imaging methods used for biomedical images in cell counting research. Most common type of imaging is CT and MRI).

4.2 Why do Cell Counting?

For in vitro cell culture, counting cells is frequently an essential but time-consuming step. Accuracy and repeatability in experiments are ensured by maintaining constant cell concentrations. Cell counts are important for evaluating immortalization or transformation,

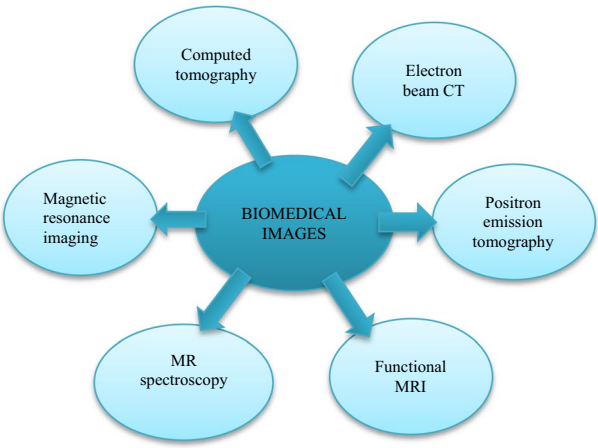

Fig. 6 Various kinds of medical imaging research

seeding cells for further studies, transfection, or infection, monitoring the health and proliferation rate of cells, and preparing for tests involving cells. For quantitative assessments of cellular responses, it is crucial that cell counts be precise, reliable, and quick. Calculating the number of cells is necessary for monitoring cell reproduction and viability, optimizing cell culture conditions, and preparing cell-based experiments. Reliable monolayers are essential for tracking cell viability and reproduction rate, assessing changes in visibility or morphology, seeding colonies for further investigations, transfection, or infections, and developing fibroblast analyses [11]. Especially for measuring cellular functions, accurate flow cytometry is crucial, consistent, and quick. Despite the importance of speed and precision in cell counting.

4.3 Benefits of Cell Counting

1. Collecting colonies is a laborious yet important process in in vitro model cultivation.
2. Maintaining constant cell levels improves the credibility and precision of experiments.
3. Inaccuracies in hemocytometers may arise from various factors: irregular handling of the specimen, fewer total cell lines in the specimen, subjective judgment regarding whether a provided cell falls within the clearly delineated counting area, contamination of the counting chamber, variability between users, and differences in the percentage of cell suspension used to fill the chamber.
4. Plotting the logarithm of Vorpal blade cell counts against the logarithm of Beckman viable cells reveals that, across all investigated cell lines, Scimitar numbers correspond to Beckman count data.

4.4 Methods for Cell Counting

The four primary methodologies employed for image segmentation, aimed at identifying, analyzing, and assessing cells and their subareas, include histogram-based, boundary-based, region-based, and pixel-based methods. Various grouping and categorization-based perceptual techniques have been demonstrated in the cancer detection process [12]. Preprocessing will involve the utilization of morphological and image filtering techniques to enhance cell images, thereby striving for optimal results.

4.4.1 Hemocytometers

The first device specifically designed for obtaining accurate cell counts was the counting chamber, also known as a hemocytometer. This chamber consists of a glass slide with a gridded pattern attached to it, where the squares are further divided into 0.05 mm x 0.05 mm squares, typically forming a 1 mm square. A designated glass coverslip is placed at the chamber's edges, creating defined zones with known volumes. Before usage, the hemocytometer should be cleaned with lens paper and 70% ethanol. Then, gently place the coverslip on top of the counting chamber, ensuring the Newton's rings phenomenon, a concentric- ring pattern of color, is observed when the coverslip is correctly positioned. A pipette is then used to collect a small sample of the cell suspension, positioning it close to the chamber's edge to allow capillary action to carry the sample into the counting chamber [13]. Before adding the cell solution to the chamber, trypan blue (in a 1:1 ratio) should be added to the cell solution to determine cell vitality.

4.4.2 Coulter Counters

Coulter counters measure electrical resistance across one or more microchannels rather than optical resistance. When cells with higher resistance than the electrolyte solution in which they are suspended pass through the channels, there is a brief increase in resistance. The Coulter counter detects this change, which increases with cell size. Coulter counters function similarly to automated cell counters. Before starting the run, the cell solution is placed into a vial after proper mixing and dilution as needed to achieve uniform cell dispersion. However, using Coulter counters requires running a blank first and then flushing the device after use, unlike automatic cell counters.

4.4.3 Flow Cytometers

Flow cytometers are commonly used for comprehensive cellular analysis, equipped with fluorescence detection technologies capable of identifying tagged intracellular components. While not all flow cytometers can count cells or measure liquid volumes, those that can, using fluorescently labeled antibodies can provide highly accurate cell counts and differentiate cells based on factors such as protein expression. Thanks to this capability, they can discriminate between cell types of the same size within the same sample or even between cell types at various stages of development. However, such complex studies complicate the experimental setup, and antibody incubations usually take hours, potentially prolonging the process significantly. Despite this complexity, using a flow cytometer is relatively straightforward (load and run), with ease of use heavily dependent on the experimental setup.

4.4.4 Spectrophotometers

Occasionally, it is possible to assess the relative density of cells using spectrophotometry. As cell density increases, less light flows through the cuvette due to turbidity caused by cells. However, because spectrophotometers measure absorbance rather than actual cell counts and other fluctuating elements of cell suspensions may affect absorbance, spectrophotometry is not a reliable method for calculating cell density [14]. To determine cell density, place the cell suspension in a cuvette and measure absorbance using a

spectrophotometer. You can compare your sample to another one if you want to assess relative density. However, to determine absolute cell density, you must compare to cell suspensions with known densities.

4.5 Biomedical Images Types and Samples

Biomedical imaging encompasses a wide array of techniques aimed at analyzing various physical measurements of the human body from different perspectives, including microscopic and macroscopic views [15]. In the field of medical science, numerous imaging techniques have been developed and utilized. Examples include X-ray radiography and fluoroscopy, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), thermography, and ultrasound imaging (USI). Many of these technologies have seen integration with soft computing and machine learning as they have advanced. Through methods like X-ray imaging, these techniques are employed to identify and diagnose health issues within the body. The following are some of the most prevalent biomedical imaging modalities.

1) CT: Computed Tomography
2) EBCT: Electron Beam CT
3) fMRI: Functional MRI; MRI, Magnetic Resonance Imaging;
4) MRS: MR Spectroscopy
5) PET: Positron Emission Tomography
6) SPECT: Single Photon Emission CT

4.6 Biomedical Images Types

Biomedical Images can be classified into three categories based upon their inherent nature as shown in Fig 7. They are macroscopic (can been seen by naked eye), light microscope and sub-microscopic.

4.6.1 Macroscopic images

These are the images that can be seen with the naked eye. These photos may be analyzed without the need of any specific equipment. A specific quantity of item is considered in the macroscopic way without events accruing at the molecular level to investigate [16]. We didn't examine changes in the substance's structure in this technique because only a few variables are required to describe the object's state. As a result, it is possible to conclude

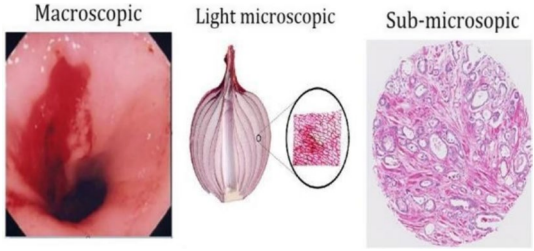

Fig. 7 Types of biomedical images for cell segmentation

that no mathematical computation is required in this technique since just the average value is used to analyze the image as shown in Fig 9. Individual cells cannot be examined using this procedure.

4.6.2 Light microscopic images

Visible light is utilized in a light microscope to see the structure of a cell in an image. The change in the conditionof individual cells, as well as their behavior and characteristics, may be easily observed using this approach. As a result,it aids in the detection of diseased and normal cells in images, which is challenging in macroscopic images. The inspection of onion cells under a microscope reveals tiny brick-like cells with nuclei, as depicted in Fig 9.

4.6.3 Sub-microscopic

A method for acquiring high-resolution images of both biological and non-biological materials is electron microscopy (EM). In medical science, it is critical for analyzing the interior structure, size, and form of cells. With the use of an integrated electron beam with a very small duration in the waveform, it produces a high-resolution picture. As seen in Fig 4, it provides precise information about the sick cell and the normal cell. So, electron microscopes employ signals generated by an electron beam's interaction with a sample to gain information about structure, morphology, and content [17]. The transmission EM (TEM) and the scanning EM (SEM) are the two forms of EM (SEM). The TEM allows researchers to see cells at very small scales and analyze living cells in a material. The transmission pathway of cells and their interior architecture are viewed and studied via TEM. The scanning electron microscope is the second type of EM (SEM). It allows you to look at the surface of the cells. It provides crucial information about the cell's whole body as well as its surface. It aids in cell counting, size inspection, and cell management in images. Electron microscopy is used for a variety of purposes, including diagnosing illness and treating patients using biopsy samples [18]. It is also used in many industries for quality control and defect investigation. EM is now employed mostly in biomedical research.

5 Literature Review

The amount, size, and placement of animal tissue have been the core focus of CIS (Cell Imaging Systems) studies since the 1960s [19]. Since the late 1990s, advancements in microscopy have made it feasible to view anti-anti entities, leading to the use of anti-anti image classification. These studies primarily discuss the nuclei of organisms. From the beginnings of CIS research, the differences between the cytoplasmic and nuclear components in terms of frequency have been explored to locate the cell's perimeter. Recent CIS research has discovered methods for recognizing microtubules and their elements. Cellular actions involve the cytoskeleton, mitochondria, and genetic material. The cytoplasm, a semi-fluid structure, contains a system of fibers that influence the shape of the cell nucleus. Various coloring treatments and approaches have been used to recognize cellular structures, including seedlings. These components help differentiate the nucleus from the background more quickly than comprehensive photographs. Investigators and counselors regularly inspect cell images for overlapped boundaries due to medication

effects or microbial attacks. To analyze these intracellular features, photos must be segmented. Current genome-wide screening initiatives use a computerized High Reliability Score (HRS) assessment. This research focuses primarily on CIS work completed since the year 2000, encompassing classification techniques in digital technology, electrophoresis in biological sciences, and histopathology. Machine learning academics are developing unique methods for identifying, analyzing, and classifying cellular components. Different elements are categorized to track cells experiencing nonlinear behavior due to mutations. Distributions are utilized to combine and select statistically significant portions. The mobility of cells has been examined using phase-contrast imaging. Additionally, revolutionary template matching techniques are employed in video microscopy to recover nuclei using moment photography.

1. Michele Perry (2022) [20] et al. In the fluorescent direction, all lattice-deceased microorganisms are tallied, while the complete cell suspension is recorded in the wide-field broadcaster. By adjusting the system configuration, picture focusing, hemocytometer spectrum, and stain conditions, precise, quick, and reliable E. coli cell counts and viable measurements were attained.
2. Ertürk (2022) et al. [21] Automated cell counting techniques like flow cytometry have been developed for faster and more accurate findings. In all flow cytometry techniques, fluid containing cells is transported through a microchannel one at a time. These techniques allow for automatic counting of cells and measurement of their physical characteristics, including size. When inspecting multiple cell types simultaneously, cells can be categorized according to their size, making it possible to calculate the number of cells in each group. A camera records the movement of cells through the microchannel, and the proposed method enables automatic detection, tracking, counting, and classification of cells based on their size.
3. You Zhou (2022) et al. [22] proposed the Erythroid Counter pipeline, which fully automates the detection and classification of erythroid cells using deep learning approaches. To assess the health condition and treatment plans of patients with leukemia or hematopathy, bone marrow erythroid cells must be detected, recognized, and counted. Standard hospital procedures rely on manual detection, chemical reagent staining, and laboratory equipment-based counting, which are time-consuming and labor- intensive. The development of deep learning in image processing allows for accurate automated erythroid cell detection and categorization.
4. Shenghua He et al. (2021) [9] suggested a novel approach for autonomously detecting cells in microscope images using distribution of possible extrapolation. Compared to previous density correlation coefficient approaches, the proposed method incorporates two advances.
5. Falko Lavitt (2021) et al. [14] Measuring cells are a crucial component of cytology in cell biology, processed in both research and practice. For example, the total cell count can evaluate whether tumor cells might proliferate under certain circumstances. However, counting cells manually is labor-intensive and time-consuming. To automate this process, the authors propose using an end-to-end trained Fully Convolutional Network (FCN). They frame the diagnostic problem as a predictive task rather than a classification task, contrary to most previous work.
6. Carina Albuquerque (2021) et al. [13] described an automated cell counting method based on deep learning using the zebrafish xenograft cancer model. This novel strategy helps comprehend tumor biology and tailor individualized treatments. The authors

implemented a customized architecture based on the Faster R-CNN with the Inception ResNet V2 feature extractor. To enhance the method, they performed several adjustments to address issues such as overlapping cells, the large number of objects to be detected, the variety in cell size and shape, and the small dataset size. This approach produced a median error of 1% in the total cell count.

7. Yuefei Lin (2021) et al. Image pre-processing is carried out using the Otsu method to establish an automatic global optimal threshold for segmentation to achieve batch counting of images. Additionally, marker watershed was used to separate adherent cells and avoid over-segmentation. The number of cells in phase-contrast microscopic pictures was calculated from the number of connected domains in the binary image. To further assess the performance of the proposed method, ImageJ was used for a comparative experiment, showing better performance in cell counting.
8. Suraj Neelakantan (2020) et al. [5] In this study, a machine learning system evaluates data input to distinguish traits and learn from them. This dissertation focuses on studying white blood cells (WBC) using deep neural networks. Together with HemoCue AB of Ängelholm, Sweden, the authors aim to create artificial intelligence for assessing white blood cells using the Uploaded R WBC DIFF Technology.
9. Weidi Xie (2018) et al. [2] The authors developed and compared settings for two fully convolutional regression networks (FCRNs). Because the connections are entirely pooling layers, they can predict a mapping population for a variable-shape input vector, which is adjusted to work effectively via end-to-end training on picture areas. The study shows that FCRNs trained entirely on datasets can provide excellent predictions on electron micrographs from specific biological experiments without merging the data. Additionally, by flipping image features, the authors demonstrate how much information an input image retains across multiple elements.
10. Yujin Zeng (2018) et al. [8] Cellphone microscopy technologies have the potential to meet various imaging and cell counting needs in health monitoring. The mobile imaging device produces results that are in great concordance with those of professional microscopes and flow cytometry.
11. Pramit Ghosh (2016) [10] et al. This project aims to provide a health device for the automated computation of WBC counts from gram-stained images. Neutrophil enumeration using cell counting offers higher accuracy than manual counting, although it requires long preparation times and expertise. Designed for use in remote areas, the device uses stained blood samples to lower costs. The proposed technique analyzes gram stain morphologies to emphasize WBCs for segmentation. The method involves rescaling the background and removing unnecessary areas from the image set.
12. Hugo Miguel (2015) [23] et al. Insufficient health and human resources, as well as time-intensive fluorescent dye serum diagnostics, hinder prompt detection and community surveillance. Several machine learning strategies have been applied to determine and characterize various biological components. The authors propose a technique for portable blood smear image segmentation and counting of red blood cells, as well as the identification of white blood cells. The method includes thresholding approaches, subcellular edge detection methodologies, inhomogeneous segmentation algorithms, and length drainage basins for red blood cells and diffusion morphometric restoration processes for white blood cells.
13. Janice Lai (2015) [24] The authors present an image processing algorithm for automatically counting and characterizing hemocytometer cells. The algorithm extracts the region of interest and identifies cells using the Canny edge detector and circular Hough transform.

14. Ivan V. Grishagin (2014) [11] By combining a regular light microscope with a video camera to gather images of dispersed cell cultures, the authors propose a comprehensive method for automated live cell detection. They developed two image- processing techniques to identify these pixels mechanically.
15. Xiaomin Guo (2013) [25] et al. This report outlines an efficient graphics-based automated cell counting method. Using a dual- distribution approach, the method separates cells from the background, fills cells using flood fill, and identifies them using blob detection. The technique's greatest benefit is its flexibility to diverse cell types and resilience to bright lights.
16. Venkatalakshmi B. (2013) et al. [15] Red blood cell count provides vital clinical information for various disorders. The reliability and efficiency of the traditional method of counting RBCs under magnification depend on the medical laboratory technologist's skill. This technique is labor-intensive. Automated hematological analyzers offer an alternative for detecting RBCs but are costlier, making them impractical for all diagnostic environments. This research proposes a fast and cost-effective method for automated red blood cell counting using graphics processing.
17. Per Jesper Sjöström (1999) [19] et al. Automating cell counting in histological preparations containing debris and synthetic materials is challenging using traditional image analysis tools, such as systems that rely on boundary contours and histogram thresholding. The authors developed an automated cell counter by combining artificial intelligence with traditional image analysis to mimic manual cell recognition.

The important key factors that have been observed during the research from year 1977 to 2022 have been shown in Fig 8 and Table 4.This table is also showing the improvement in cell counting from last two decades.

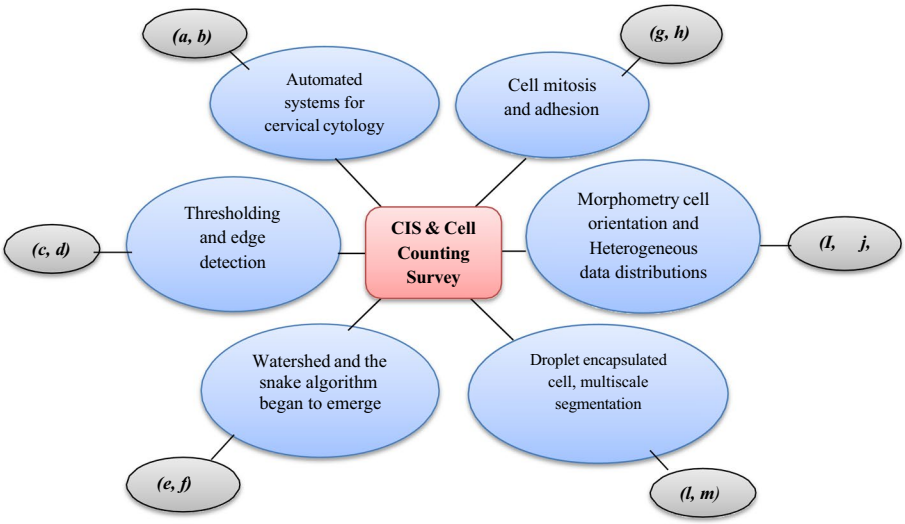

Fig. 8 **a** Aus et al. (1977) [34], **b** Abmayr et al. (1979) [35], **c** Gorman et al. (1985) [36], **d** Harms et al. (1986) [37], **e** Thiran et al. (1996) [38], **f** Young et al. (1998) [39], **g** Zimmer et al. (2002) [56], **h** Schmitt and Hasse (2008) [40], **i** Huang et al. (2018) [41], **j** Falk et al. (2019) [42], **k** Van et al. (2019) [43], **l** Lv et al. (2020) [44], **m** Jingwen et al. (2020) [45]

Table 4 Research scope for various decades for cell segmentation

Year	Research
1970's	For cervical cytology, computerized prescreening devices [34, 35] were used
1980's	Edge detection and thresholding [36, 37]
1990's	The snake algorithm and Watershed first appeared [38, 39]
2000's	Cell adhesion and mitosis are two examples of object motion, collisions, breakage, and merging [56, 40]
2010's	Object count, morphology recognition, cell orientations, and mitogenic dynamics learning systems [41, 42]. For training and testing, centralized data distributions [43]
2020's	Cell segmentation using multistate hybrid models and segmentation of droplet-encapsulated cells [44, 45]

This article shows the comparative and full details of the various parameters used for cell counting and image segmentation shown in Table 5. It conations different parameters used in different articles, such as input, output, dataset used, techniques followed and future scope. This table explores state-of-the-art studies in literature to cell counting methods.

6 Algorithms Used for Cell Counting and Cell Segmentation

Figure 9 illustrates various algorithms used for cell counting based on Deep Learning, Machine Learning and Soft Computing. It is highlighting their roles and methodologies. The figure highlights convolutional neural networks (CNNs), which are adept at identifying intricate patterns in cell images through deep learning. The watershed algorithm is shown for its effectiveness in segmenting overlapping cells by treating the image as a topographic surface [26]. Additionally, fuzzy logic methods are included for handling imprecision in cell boundaries, and thresholding techniques like Otsu's method are noted for determining optimal segmentation thresholds. Active contour models (snakes) are also featured for delineating cell boundaries by evolving curves based on image gradients.

6.1 Deep Learning Techniques

Deep learning strategies have recently been extensively used for cell image segmentation. These strategies focus on training classifier systems to be used in various applications. The classifier is trained by automatically extracting and selecting the features of the region of interest (ROI). These methods have proven to be more efficient compared to many other machine learning methods. However, they require a large amount of data for the training dataset [27]. Hence, the use of this approach is limited to real-world cases because finding large datasets that are openly available is a tedious job. M. Freiman et al. proposed a new and almost fully automatic approach for the segmentation of liver tumors [28]. An SVM (Support Vector Machine) classifier is used to classify healthy cells and tumor cells from CT images, which leads to the generation of a new set of high-quality seeds. This suggested approach is efficient, robust, and comparable to, if not more effective than, many other semi-automatic strategies. Authors in [29] proposed a fully automatic segmentation algorithm to detect tumors in livers from CT images of patients. The novelty of this approach was combining follow-up- based detection with convolutional neural networks

Table 5 Literature survey based on various research articles

Ref.no	Input	Technique followed	Name of dataset/types of images	Types of cell	Output/performance	Limitations/future scope
[1]	Cell enumeration	To directly count E, a chip-based image cytometer employs a thin-gap, low volume counting chamber consumable	Bacterial cells	Bacterial cells	Image cytometer may yield a variety of data metrics, including total, live, and dead cell concentrations, viability, and cell size, which can provide additional information about the health of the cells	The photo cytometer technique, in comparison, can produce multiple data parameters, including total, live, and dead cell densities, viability, and larger cells may provide more information about the health of the cells
[2]	Coating the collected particles onto glass sliders	Cytometry using a Fossomatic FC Cell	Microscopic images	Blood cells in blood smear	The results of the mobile phone microscope are consistent with those of Commercial microscopy and flow cytometry. Because of improvements in miniaturized circuits and cellphones, this economical, portable smartphone microscopic platform may be utilized as typical and practical instruments to carry out actual point-of-care biomedical or biochemical Lab-on-a-chip applications	Due to the importance of micron size atom counting in clinical diagnosis and the demands for low-cost and time-efficient solutions for point-of-care applications, this innovative layout of a low-cost and compact mobile phone microscopic device and its particle/cell counting performance had demonstrated its potential in future point-of-care and biological applications at home or in underdeveloped areas

Table 5 (continued)

Ref.no	Input	Technique followed	Name of dataset/types of images	Types of cell	Output/performance	Limitations/future scope
[3]	Microscopy images	Density regression-based method	Microscopy images	Blood cell	In terms of MAE and MRE, the suggested method outperforms the other eight methods in terms of cell counting performance	The suggested method is used with a made-up dataset. It is possible to implement it on a real-time dataset that might be used for real-time clinical applications. Additionally, it has the potential to be used to solve a variety of issues, including the counting of objects (other than cells) in crowded environments
[4]	White blood cell	Hemocytomet er Hybrid (Fuzzy logic + Random forest)	Microscopic image	Blood smear cell	The system's sensitivity and specificity were determined to be 96.4% and 79.6%, respectively, using a database of 150 blood smear slides obtained from various health centers of the Kolkata Municipal Corporation in Kolkata, India	Using pictures obtained with fluorescence microscopes, which will help to eliminate artifacts [35] in the captured images, will increase the system's performance even further. A full disease map for each region and year may be created using GPS
[5]	Attaching a web camera to a conventional microscope	Hemocytomet er	ImageJ	U251 cells	When compared to hand counting, this method is about ten times quicker and produces more trustworthy and consistent results	When compared to hand counting, this procedure is around 10 times quicker and gives more reliable and consistent results

Table 5 (continued)

Ref.no	Input	Technique followed	Name of dataset/types of images	Types of cell	Output/performance	Limitations/future scope
[6]	Microscopy-based blood diagnosis is impediments to timely diagnosis and population	Image processing	Techniques for adaptive thresholding selection	Transform-based red blood cell watershed and gradient-restricted morphological reconstruction	The classification findings show that this technique is capable of providing estimates of the quantity of red blood cells, accurately extracting white blood cell candidates, and accurately differentiating them in either mono-or polymorphonuclear with the examined properties	In order to make fair performance comparisons between these and previously developed methodologies, the algorithms developed for both image processing methodologies should be tested in databases of high-quality images, and vice versa (the performance of the state-of-the-art algorithms in low-quality images should be assessed). The current version has a few memory leak issues
[7]	Cell morphology,	K-means clustering method segment	Microscopic image	Microscopic image of blood cell	Regardless of cell shape, cell type, or cell size, testing findings show that the counter can identify the number of cells rapidly and correctly while maintaining good stability	It has no ability to recognize and discriminate between separate cells in a normal picture

Table 5 (continued)

Ref.no	Input	Technique followed	Name of dataset/types of images	Types of cell	Output/performance	Limitations/future scope
[8]	Pixel data	Convolution Neural network	Microscopic images	Image data of cells	Mean absolute error and standard deviations for cell counting in a typical synthetic cell data-set	Mean absolute error and standard deviations for cell counting in a typical synthetic cell data-set. Little modifications to these real-world photographs can improve performance even further
[9]	Dense cell microscope images	Deep learning and transfer learning CNN	Microscope images of human cancer cell lines	Microscopic image	It obtains a test mean absolute error of 12 compared to 32 (33) produced by deep learning without transfer learning and 41 (37) acquired by the best machine learning pipeline (15). (Random Forest Regression using Gradient Histogram features)	The suggested method can handle dense cell microscope pictures of two unique cell lines, such as human osteosarcoma (U2OS) and human leukemia (HL-60), as well as real-world data with varying degrees of light, occlusion, appearance differences, and other characteristics
[10]	Blood cell images	Hough Transform	Red blood cell	Blood cell	To be successful and economical, the automated red blood cell counting method presented in this study employs image-based analysis	This computer-based automated red blood cell counting approach will be used to real-time microscopic pictures taken from a digital Camera mounted to a microscope

Table 5 (continued)

Ref.no	Input	Technique followed	Name of dataset/types of images	Types of cell	Output/performance	Limitations/future scope
[11]	Blood samples	Segmentation algorithms	Microscopic Images	Bone marrow images	The system accurately segments densely crowded leukocytes in bone marrow pictures, according to our testing data	Our data show that the algorithm almost always accurately locates each cell. As a consequence, the method enables picture interpretation, as well as the first computer- based appraisal of bone marrow samples
[12]	Cell images	Cell Segmentation	ImageJ	Image Cytometry		Authoritarian platforms with open access are essential for scholars all around the world to exchange, study, and teach data, codes, and algorithms
[13]	Blood cells images	Deep learning techniques CNN	White blood cells	White blood cells	With a total accuracy of 95.45% in WBC identification and 90.49% in WBC differential classification, the CNN Classifier has shown extremely encouraging results	Several photos of blood and its constituent parts are now available because to the advancement of haematology. Convolutional neural networks and diverse design methodologies in CNNs are powerful tools at our disposal

Table 5 (continued)

Ref.no	Input	Technique followed	Name of dataset/types of images	Types of cell	Output/performance	Limitations/future scope
[14]	Intrinsic	Cancer Crusade	Cancer cell	Cell Metastasis		We provide our perspective on the metastatic process and suggest prospective future research avenues, relying on new mechanistic insights from recent studies
[15]	Overlapping cells images	Deep learning-based cell detection R-CNN	Inception ResNet V2	Cancer cells	This technique's median error was over 1% of the total number of cell units. Our method boosted the average accuracy from 71 to 85% on the tested data set	Future study will seek to adapt this strategy other cell types that have similar problems. The insights acquired from this experiment will ultimately be used to a new, larger data set to generate more accurate models
[16]	Blood cells	Segmentation method	Microchannel images	blood cells	The proposed approach has been evaluated in a variety of trials, and it has been determined that cells can be spotted accurately using image processing methods, and cell counts can be conducted effectively up to a specific flow rate	In the future, camera photos will be taken straight from the camera's serial port and processed online. Total computing time can therefore be reduced when the amount of file read time is reduced

Table 5 (continued)

Ref.no	Input	Technique followed	Name of dataset/types of images	Types of cell	Output/performance	Limitations/future scope
[17]	Cells in phase-contrast microscopic images	Segmentation method	Microscopic images		The experimental findings revealed that the suggested method's average values of MIS, INC, and ACC are only 3.31%, 3.49%, and 96.69%, respectively. Furthermore, each cell picture was tallied in an average of 0.65 s	Because it is simple, practical, rapid, and extremely accurate, the recommended cell counting approach may be utilised successfully for cell counting of phase-contrast microscopic pictures
[18]	Synthetic materials	Artificial Neural Network	Aided Image Analysis	NIH Image	The correlation measure at 100×magnification was close to person-to-person variability, but not meaningful at 50×magnification. The machine was around six times quicker than a skilled person	Lastly, hand counting is time-consuming, inefficient, and costly. Freeing up workers for more difficult jobs is a significant technical achievement in the histology laboratory

Table 5 (continued)

Ref.no	Input	Technique followed	Name of dataset/types of images	Types of cell	Output/performance	Limitations/future scope
[19]	Laboratory equipment	Deep learning method	Images, cell densities, and cell position	Bone marrow erythroid cells	The Erythroid Counter effectively detected erythroid cells with a precision of 87.16%, recall of 87.45%, accuracy of 86.33%, and F1 score of 87.30%. ErythroidCounter recognised erythroid cells with an accuracy of 90.6%, recall of 90.2%, and F1 score of 90.8%	Furthermore, we will continue to improve our model to make it more suitable for categorizing pictures of erythroid cells and to improve cell classification precision
[20]	Microbial cells	Thresholding based algorithms	Synthetic Images	ImageJ	Overall, we determined that Bernsen local thresholding produces the best results, including cell counts and morphological analysis	The Bernsen technique can be improved by modifying the threshold selection procedure [20]

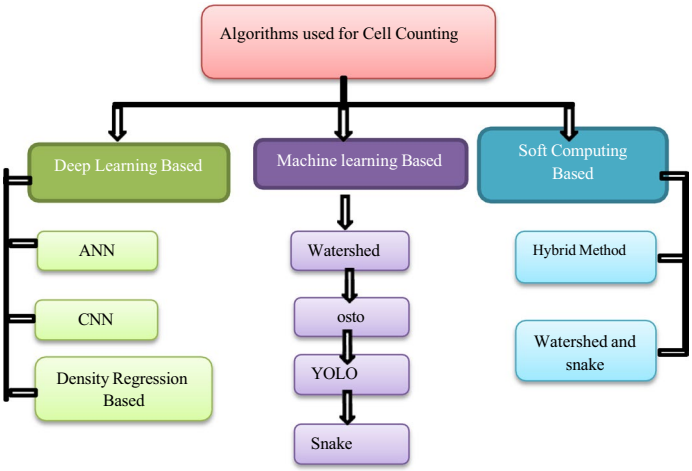

Fig. 9 Cell counting algorithm

(CNNs). The CNN is trained to construct a voxel classifier with automatic feature learning. This approach is more effective and efficient in terms of accuracy, showing an improvement of 60.29% compared to other methods.

6.1.1 CNN (Convolutional Neural Networks)

Convolutional Neural Networks (CNNs) [30, 31] are highly effective for cell counting in biomedical images. They automate the detection and counting of cells in microscopic images, significantly reducing manual effort. CNNs can accurately segment and classify cells, even in dense and overlapping conditions. By using layers of convolutional filters, CNNs extract relevant features such as cell edges and textures. After preprocessing the images, a CNN model is trained on labeled datasets to learn distinguishing cell characteristics. Post-training, the model can analyze new images to identify and count cells. Advanced CNN architectures, like U-Net, enhance segmentation performance for more precise cell counts. This technology is crucial for applications in medical diagnostics, research, and drug development, providing reliable and reproducible results.

6.1.2 ANN (Artificial Neural Networks)

Artificial Neural Networks (ANNs) can be utilized for cell counting in biomedical images, though they are less specialized than CNNs for image-based tasks. ANNs consist of interconnected layers of neurons that can learn complex patterns from data. For cell counting, ANNs are trained on features extracted from images, such as shape, size, and intensity of cells. Preprocessing involves converting images into feature vectors that the ANN can process[31]. The network is trained on labeled data, learning to associate specific features with cell counts. While ANNs can perform this task, they typically require extensive feature engineering. ANNs can be combined with other techniques to improve accuracy. Despite their versatility, ANNs are generally outperformed by CNNs in image analysis tasks due to CNNs' ability to automatically learn spatial hierarchies. However, ANNs remain useful

in simpler or complementary roles in cell counting applications, contributing to advancements in medical research and diagnostics.

6.1.3 Density Regression Based Model

Density regression-based models for cell counting offer an effective approach to handle the challenges of densely packed and overlapping cells in biomedical images. Instead of detecting individual cells, these models estimate a density map where the integral over a region gives the count of cells. The method involves training a regression model, often a Convolutional Neural Network (CNN), to predict these density maps from input images. During training, the model learns to map image features to the corresponding density values. This approach is particularly advantageous in scenarios with highly clustered cells, where traditional detection methods struggle. It bypasses the need for precise localization, focusing instead on accurate cell density estimation. Density regression models are robust against variations in cell size and shape [32]. They provide a continuous and smooth estimation, making them suitable for real-time and large-scale analyses. This technique is increasingly applied in biological research, pathology, and drug development, enhancing the accuracy and efficiency of cell counting processes.

6.2 Machine learning based method

Machine learning has significantly advanced the field of cell counting by automating and enhancing the accuracy of the process. Techniques like convolutional neural networks (CNNs) are particularly effective, as they can learn and identify complex patterns in cell images. These methods facilitate the classification and counting of various cell types, even in large and diverse datasets. Machine learning algorithms can handle challenges such as overlapping cells, varying cell shapes, and differing sizes [33]. Automated systems using machine learning reduce the time and labor required for manual counting, providing faster and more reliable results. Applications range from medical diagnostics to biological research, where precise cell counts are critical. Additionally, innovations like deep learning and support vector machines (SVMs) have improved the segmentation and identification of cells, leading to more robust and scalable solutions [34].

6.2.1 Watershed Algorithm for Cell Counting

The watershed algorithm is a classical image segmentation technique used for cell counting, particularly effective for separating closely packed cells. It treats the grayscale image as a topographic surface, where the brightness represents elevation. By simulating the flooding process from local minima (markers), the algorithm segments the image into distinct regions corresponding to individual cells. This method is particularly useful in scenarios with overlapping cells [35]. However, it requires precise markers and can be sensitive to noise, making preprocessing steps like filtering and morphological operations crucial for optimal performance.

6.2.2 YOLO (You Only Look Once) for Cell Counting

YOLO is a real-time object detection algorithm that can be adapted for cell counting. YOLO treats cell detection as a single regression problem, directly predicting bounding

boxes and class probabilities from entire images in one evaluation. It divides the image into a grid and assigns bounding boxes to cells, providing high-speed detection suitable for real-time applications [36]. YOLO's efficiency and accuracy make it a powerful tool for counting cells in large datasets, though it may struggle with extremely dense or overlapping cells without modifications [35].

6.2.3 Snake Algorithm for Cell Counting

The snake algorithm, or active contour model, is a segmentation technique used for delineating cell boundaries in images. It involves initializing a contour around each cell, which then iteratively adjusts to fit the cell's edges based on image gradients and internal energy constraints [37]. This method excels at accurately tracing the shapes of individual cells, particularly when they have well-defined edges. However, it can be computationally intensive and may require careful tuning of parameters. It is effective for counting cells with distinct boundaries but less so in highly cluttered environments [38].

6.2.4 Otsu's Algorithm for Cell Counting

Otsu's algorithm is a thresholding method that separates cells from the background in an image by maximizing inter-class variance. It automatically determines the optimal threshold value to convert a grayscale image into a binary image, where cells are distinguished from the background [39]. This method is simple and fast, making it suitable for initial segmentation stages. While effective for images with clear contrast between cells and background, Otsu's algorithm may struggle with images that have variable illumination or low contrast, necessitating additional preprocessing for accurate cell counting.

6.3 Soft Computing

Soft computing is an emerging field in computer science that tackles imprecise and uncertain problems, aiming to find approximate, robust, and cost-effective solutions. Its ultimate goal is to emulate the human mind as closely as possible. The shift from hard to soft computing is largely influenced by the limitations of traditional mathematical calculations, which can make some problems nearly unsolvable or overly complex [44[. Soft computing includes approaches like evolutionary computing, artificial neural networks, fuzzy logic, and Bayesian statistics. These methods can be used individually, but one of the main advantages of soft computing is the integration of multiple methods, which can provide more comprehensive solutions to complex problems. There are two major advantages to using soft computing. First, it simplifies the resolution of non-linear problems for which traditional mathematical methods are unavailable. Second, it incorporates human-like cognitive abilities such as recognition, perception, and learning into computing [40]. This integration allows for the development of intelligent systems like autonomous self-tuning systems and automated design systems. Professor Lotfi Zadeh, who also developed the concept of fuzzy logic, first introduced the term "soft computing". Soft computing is applicable to both artificial and natural problems, and its role model is the human mind. It encompasses a variety of computational techniques used in artificial intelligence and machine learning, and is applied in engineering fields such as aircraft, spacecraft, HVAC systems, communication networks, mobile robotics, inverters and converters, electric power systems, power electronics, and motion control[41].

6.3.1 Hybrid Methods for Cell Counting

Combine multiple techniques to leverage their individual strengths and mitigate their weaknesses. These methods integrate approaches like convolutional neural networks (CNNs) for feature extraction, classical algorithms like the watershed for segmentation, and machine learning models for density estimation. For instance, a hybrid approach might use a CNN to generate a probability map of cell locations, followed by the watershed algorithm to delineate individual cells accurately [42]. Combining deep learning with traditional image processing ensures robust performance across varied and challenging conditions, such as overlapping cells and diverse cell shapes. Hybrid methods can also incorporate post-processing steps, like morphological operations, to refine segmentation results [43]. These approaches offer improved accuracy and reliability over single-method solutions, making them ideal for complex biomedical imaging tasks. By harnessing the power of both advanced and classical techniques, hybrid methods provide comprehensive solutions for automated cell counting, enhancing the precision and efficiency of biomedical research and diagnostics. The comparison between various segmentation techniques have been shown in Table 6.

6.4 Cell Image Segmentation

Cell is the basic, biological, and operational part of every living bodily parts and it comes in a variety of sizes, shapes, and functions [44]. Segmenting a cell image is the method of identifying cellular components or things inside a photograph. It is a crucial stage in the interpretation of physiological images. It necessitates segmenting or dividing animage's pixels into regions of interest (ROI), as seen in Fig 10. A wide range of research domains, such as drug development and the study of cellular dynamics in both healthy and unhealthy conditions connected to investigations into small parts, image extraction, and cell research. Fluorescence microscopy has advanced recently, making accurate representations of cells and their internal architecture possible.

Cytologists, histologists, embryologists, and cell biologists to analyze the shape, size, and type of cells and cellular processes use imaging technologies [45]. The two most recent developments in CIS are (a) algorithms, namely the watershed and snake algorithms, and (b) soft computing, specifically neural computing. Nowadays, a hybrid system is used that combines these two methods with soft computing. Around ten years ago, cellular activity analyzed using an electronic microscope. The conventional microscope instruments did not yield good high-resolution images. High quality images now make it reasonably easy to see a cell count, activity, and cell mitosis, as shown in Fig 11. Thus, photogrammetry increases the speed and precision of object detection at a low cost. A new approach for gathering and analyzingcellular structure in a set of patients must be developed because the population is growing daily [46].

One important use case for automated cell segmentation is mitosis, or cell division. [47]. Cell division provides accurate information about several fundamental topics, such as the severity of the illness. What is the illness's diagnosis? To what extent is the sickness spreading? To what extent do the cells react to the treatment? A microscope that uses light and electrons to study cell division in detail generates energy. The promise of throughput remains unfulfilled for human physicists and therapists until automated analysis becomes available. It has been shown that systems for capturing and analyzing cell pictures are

Table 6 Comparison of cell counting techniques

S.No	Technique	Description	Advantages	Disadvantages
1	CNN (Convolutional Neural Networks)	Utilizes data to identify and segment complex patterns with precision	High accuracy, automatic feature extraction	Requires large datasets, computationally intensive
2	Watershed Algorithm	Uses a topographic map approach to segment overlapping cells	Good for separating touching cells	Sensitive to noise, over- segmentation
3	Fuzzy Logic	Manages uncertainty and imprecision in cell boundaries	Flexible, mimics human reasoning	May require extensive tuning
4	Thresholding (Otsu's Method)	Automatically finds optimal thresholds for segmentation	Simple, fast, effective for high contrast images	Not suitable for images with varying lighting
5	Active Contour Models (Snakes)	Adjusts contours dynamically to fit cell edges accurately	Accurate boundary detection	Can be sensitive to initial position and noise

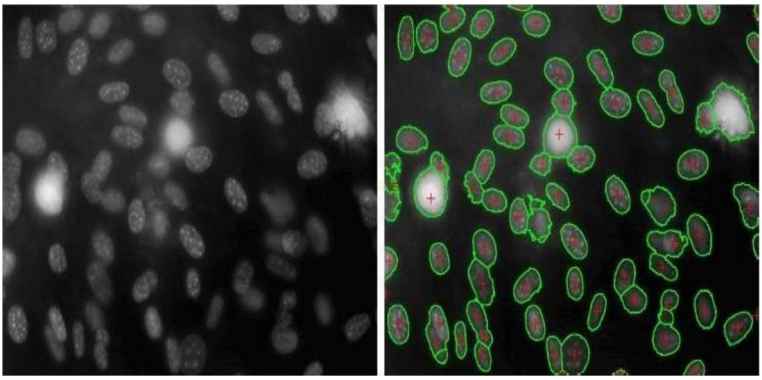

Fig. 10 A fluorescence microscope image (left) and the outputs of a segmentation cell (right). The edges of the designated squares, as well as the cell center of gravity of the actual truth have shown by emerald circles as well as red minus signs, correspondingly

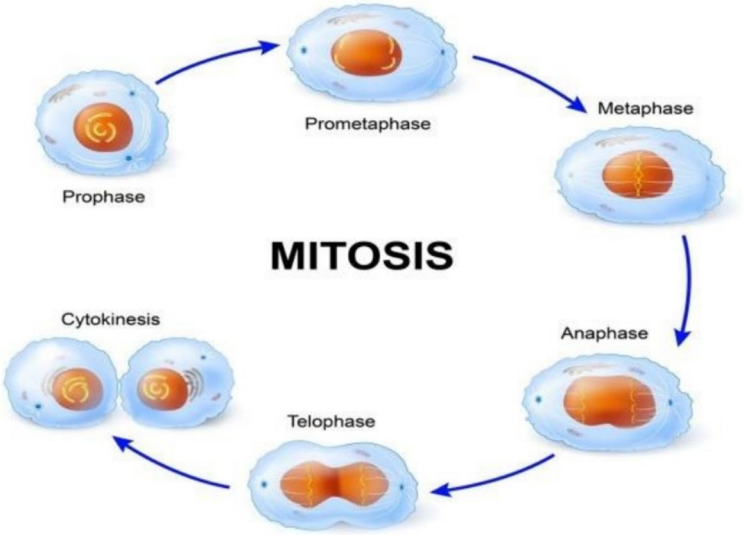

Fig. 11 Mitosis Poses a Challenge for the CIS (source internet)

widely employed in biomedical research for training, diagnosis, and therapy of diseases. It is difficult to obtain precise data for cell morphological behavior from dynamic microscopic images because of the undesired noise and brightness fluctuations in the picture [48]. The following are the three primary goals of an information retrieval and storage system for images:

- o The system for managing experimental data I/O.
- o Verify that there are no brightness or color errors.
- o Expert replication for a cell glossary.

There are *four* important methods are used for image segmentation in terms of identifying, quantifying and analyzing thecells and their sub parts namely: histogram-based, boundary-based, region-based, and pixel-based methods shown in Fig 12. There are other perceptive methods based on clustering and classification has been noted in diagnosis of cancer. The image filtration and morphological process has used to sharp the cell image for get accurate result is known as pre-processing.

6.4.1 Histogram-based Techniques

This method deals with image histogram where the existence of pixels has counted. After that the histogram is divided intosub (m) parts, p1, p2, p3….and pm. An image consist numbers of pixels that are connected with each other by same characteristics. So the detection of same region and other of pixels then grouping these pixels is also done in histogram-based technique. This task is done with threshold value that is why is also known as thresholding technique [49]. The division may be done by colors, gray-level deviation, 2D, 3D or structural equity. Most of the image processing techniques work on color/grey-level pixels in binary by using threshold value. Thresholding is a technique to compress and make image in a simple form as is required for segmentation. Threshold process is complicated when applied on 3D image buteasy in 1D[43]. There are any number of thresholds may be used in a histogram- based method. In some case threshold is more required. Basically there are two main reasons to adopt threshold method [50]. Firstly, inequity amongst object and background only main objective of segmentation like in cell image segmentation. Secondly, some methods are used to handle alteration and iterate of threshold value in histogram method for image analysis that are not developed for detecting multiple thresholds

6.4.2 Boundary-Based Techniques

Several applications are used in biomedical science to identify different items in images. Edge detection can be used sinceevery object has an edge that connects it to the boundary. A group of pixels known as an edge forms the border between two sections [51]. Edge detection can be done by using first derivative and second derivative step. First derivative tells us where the edge in an image is and second derivative tells the direction of edge as edge going from black to white or vice versa. So both palyimportant role in boundary based method. It has been noted that derivative based edge detection is extremely

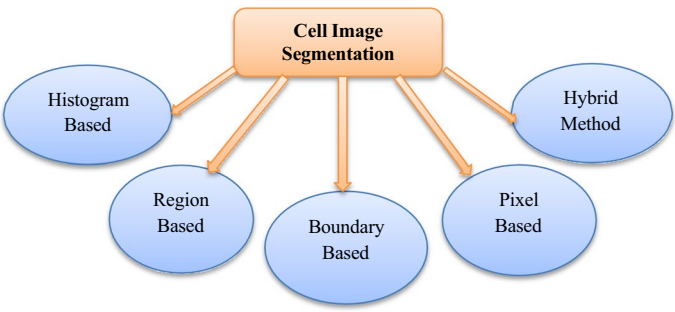

Fig. 12 Image Segmentation Algorithms

sensitive to noise.So here is need to apply special filter to remove extra noise. The edge detection shows the edge information and the relationship between pixels in an image. If pixel of image has grayvalue it means not an edge at that point [52]. However, if a pixel has a neighbor with widely varying gray level it may be an edge point. In general edges are caused by change in color and texture in image or specific lighting condition present during image acquisition process.

6.4.3 Region-based techniques

The goal of segmentation in this method is to divide an image into areas. We solved this issue by identifying regional borders based on gray-level discontinuities. Moreover, it is done using a threshold depending on the distribution of pixel characteristics like color or gray scale values. Growing-and-merging and splitting-and-merging are two ways the region-based method is used. More recently, region-based strategies have also relied on morphological procedures [53]. The region-growing mechanism divides pixels or smaller regions into larger regions based on predefined parameters. Grow areas are made by combining surrounding pixels with each seed that have the same properties as the seed being utilized, starting with a collectionof "seed" points. The nature of the problem is frequently taken into consideration when choosing a set of one or more starting points. The seed is chosen based on an image's characteristics, such as whether the grey level is high or low. Once more seedsare added, this process is repeated until no more seeds are visible [54].

6.4.4 Pixel-based Techniques

In this, the image segmentation has been done in terms of making clusters of pixels in two groups that is foreground (cell) and background pixels. The pixels that belong to same group grouped together for making one region[55]. Final output after performing the segmentation is come in the form of fore-/background significant. Selecting of algorithm that works on pixelsof image and analyze, whether one can use statistical or neural computing-based learning techniques here the core idea behindpixel-based methods is intensity. Red, blue, and green are the three principal channels that are commonly used to quantify intensity for color images , while gray scale images simply employ the gray-level. The brief comparison based on various parameters used for image segmentation by different segmentation techniques have been shown in table 7.

6.5 Hybrid Cell Image-Segmentation Systems

After the discussion of the above four methods two main point arise here, if we want to make a system based on cell image segmentation. First is we have a huge knowledge about virtual cell features and its relative intensity in a segmentation system. Second is that multiple technique based analysis for flexible accommodation of all complication. The design of a hybrid segmentation offers two choices: (a) Image experts work with computer scientists to examine segmentation on an exemplar set of images to techniques/parameter tuning. (b) Autonomous neural/machine learning from an exemplar set of images and other information collateral (Table8 represents comprehensive view of various cell counting methods in literature along with some new methods).

Table 7 Comparison of segmentation techniques

Parameters	Thresholding	Edge segmentation	Region based Segmentation	Pixel-based segmentation
Performance	Best	Excellent	Good	Good
Color space	HIS images, combination of YIQ, values and RGB and grayscale	Gray levels and RGB images	Gray levels and RGB images, intensity and saturation	Gray levels and RGB images, intensity
Segment level	Homogeneity	Discontinuity in homogeneity	Homogeneity	Homogeneity
Segmentation effect	Good	Average	Normal	Normal
Complexity	Very low	Average	High	Average
Quality measurement	Depends value upon threshold	Depends upon intensity variation	Depend upon same pixels in region	Depends upon no of pixels in cluster

Table 8 Some new methods suggested for cell counting and image segmentation in ML and DL

Technique	Description		
	Advantages	Disadvantages	New method suggested
Thresholding	1. Easy to implement 2. Less computational cost 3. Less memory space 4. High processing speed	1. Sensitive to noise and grayscale unevenness 2. Results aren't correct while there's is overlap of grayscale values	Otsu thresholding
Edge segmentation detection	1. Preserves the boundaries of images with high contra Preserves the limits of photos with excessive comparison st	1. Manual intervention is required 2. Cannot feel noise	NA
Region based segmentation techniques	1. Easy to implement 2. Noise resistant	1. Manual intervention is required 2. High computational cost in terms of time and memory	Hemitropic region-growing algorithm [22]
Pixel based/ Clustering techniques	1. Easy to implement 2. Low computational cost	1. Very susceptible to noise 2. Membership function calculation is difficult	FCM with advanced optimization techniques
Watershed Segmentation	1. Simple and intuitive 2. Algorithm can be parallelized 3. Results in complete division of image	1. Over segmentation 2. Manual intervention is required 3. Sensitivity to noise 4. Poor detection of significant areas with low contrast boundaries	Marker controlled watershed segmentation
Partial differential equations	1. Fastest computation	1. Accurate initialization and Implementable functions required	NA
Artificial neural network	1. Fast segmentation technique and hence less run time complexity 2. High results w.r.t sensitivity and accuracy	1. Time wastage during training phase 2. Works well with large ROI	Deep learning techniques

7 Applications of Automatic Cell Counting.

1. Cell counting is a popular technique and a crucial component of all cell-related research, including biological applications, genetics, biochemistry, and medical diagnostics and therapies.
2. Additionally, cell counts are crucial for preparing cells for cell-based experiments, transfection or infection, and monitoring cell health and proliferation rates.
3. Precise, dependable, and timely cell counts are critical, especially for quantitative assessments of cellular responses. While establishing a medical diagnosis, for example, the amount of leukocytes, or white blood cells, in the bloodindicates if a disease is present in the body.
4. Neutrophils, the most prevalent type of leukocyte subtype, defend the body from bacterial infections. Similar to this, another subtype, lymphocytes, defend the body from viral illnesses. The ratio of these white blood cells in the blood can be used to identify the disease's origin.
5. For another illustration, biologists must count the cells in the cell culture they use for their bacterial experiments to examine the time-dependent growth in the number of cells.
6. In short, cell-counting techniques have been developed and used for many purposes.
7. Cell counting is an important aspect of biological research. Manual cell counting, on the other hand, is time- consuming, inefficient, and has a high counting error rate.
8. For patients with abnormal blood cells, blood counts.
9. Counting sperm.
10. When sub culturing or tracking the development of cells over time.
11. Processing cells for downstream analysis: numerous tests call for precise cell numbers (PCR, flow cytometry).
12. Cell size measurements: Inferring the true cell size of a microphage by scaling it to the width of a hemocytometersquare

8 Conclusion and Future Work

The preliminary paper presentsthe most recent research on cell identification, cell counting, and deep learning algorithms. Then, in order to comprehend the various types and variations of cell images, the eminent datasets that disprove cell-counting concepts are also discussed .The major focus of this research is on current advances in deep learning and machine learning systems for Cell Counting and Image Segmentation. There is alsoan overview of the numerous uses of medical images. The principles of machine learning are also covered in order to understand the distinctions between classic machine learning and modern deep learning techniques. We are living in an excitingera for medical image processing, with endless prospects for innovation and enhancing the present state-of-the-art methods, as well as using the ability of deep learning for cell counting to have a big influence on health care across the board. Finally,a thorough analysis of real-time applications is performed, including cell detection, cell image segmentation, edge, cell courting, etc.

Proactive research and development has enabled consumers to get better outcomes faster and with less expertise than previously possible techniques, freeing up expert time for the most challenging instances. It is also worth noting that the current research emphasizes

the use of the cell counting approaches with both classical and deep learning detectors. The advancements in recognizing cells from images and their sub-modules are discussed by assessing the advantages and limitations of current algorithms and methods. Various of image segmentation techniques has been explored and varuious cell counting method has been discussed. It has been concluded from current review that hybrid method is more efficient for automatic cell counting and cell image segmentation. Finally,a thorough analysis of real-time applications is performed, including cell detection, cell image segmentation, edge, cell courting, etc. Future directions for machine learning-based detection and counting from cell images are summarized from the comprehensive study. Some of the important key challenges and research gaps have been identified, such as Different staining, morphological diversity , differentiation between cells and debris and cell overlapping etc which can be addressed while developing cell counting techniques and applications for medical image analysis in a clinical setting. Existing methods are not able to resolve these limitations efficiently. So on the basis of this review it has been observed that these factors should be considered for improvements for better and high accuracy result in future. Additionally, real-time cell counting and segmentation systems, integrated with advanced hardware and software solutions, can revolutionize clinical practices by providing immediate and accurate diagnostic results. Collaborations between computational scientists and biologists will be essential to ensure that these technological advancements are effectively translated into practical applications. The exploration of novel algorithms and hybrid approaches combining classical image processing techniques with deep learning will also be pivotal in overcoming current limitations and achieving higher precision in cell analysis.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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