

# AI- Based Cell Counting System

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**Abstract:** Accurate cell counting in biomedical microscopy is a critical task for numerous applications, including disease diagnosis, drug testing, and tissue engineering. Traditional image processing methods, while computationally efficient, often struggle with complex cell morphologies, overlapping structures, and varying illumination conditions. Conversely, deep learning-based approaches can achieve high accuracy but require large annotated datasets and are computationally expensive. In this study, we propose a hybrid vision-based framework that combines classical image processing techniques with AI driven regression modelling to achieve precise and efficient cell counting. The method first employs conventional image enhancement, segmentation, and morphological operations to extract robust low-level features such as cell boundaries and regions of interest. These extracted features are then input into a regression-based learning model, trained to predict cell counts while compensating for segmentation errors and occlusions. Experimental results on multiple biomedical microscopy datasets demonstrate that the proposed hybrid approach outperforms purely classical or deep learning methods in terms of accuracy, generalization, and computational cost. This work highlights the potential of hybrid vision systems for reliable quantitative analysis in biomedical imaging.

**keywords:** Cell counting; Biomedical microscopy; Hybrid vision system; Image processing; Artificial intelligence; Regression model; Feature extraction; Image segmentation; Machine learning; Quantitative Bio-imaging.

## 1. INTRODUCTION

Cell counting is one of the most essential tasks in biomedical and clinical research, providing quantitative information for applications such as disease diagnosis, drug screening, tissue culture monitoring, and cancer analysis. Accurate estimation of cell numbers helps researchers evaluate cell growth, viability, and response to treatments. Traditionally, this process has been performed manually by trained experts under a microscope. Although manual counting can be precise for small datasets, it is labor-intensive, time-consuming, and prone to human errors, especially when dealing with dense, overlapping, or irregularly shaped cells. To overcome these challenges, various automated image-based cell counting methods have been developed. Conventional or classical image processing techniques such as thresholding, filtering, morphological operations, and segmentation have been widely used due to their simplicity, interpretability, and low computational demand. However, their performance often degrades in complex microscopy conditions, such as non-uniform lighting, cell clustering, or noise, which make accurate segmentation difficult. With the advancement of artificial intelligence (AI), particularly machine learning (ML) and deep learning (DL), data-driven methods have shown remarkable improvements in visual recognition tasks, including biomedical image analysis. AI models can learn complex visual patterns directly from data and adapt to variability in cell shapes and imaging conditions. Nevertheless, these approaches typically require large annotated datasets and extensive computational resources, and may lack transparency and generalization when applied to unseen data. To address these limitations, this study proposes a hybrid vision-based framework that integrates classical image processing with AI-based regression for precise cell counting in biomedical microscopy images. In the proposed method, classical image processing is first employed to enhance image quality, reduce noise, and extract informative features such as cell boundaries and regions of interest.

These extracted features are then used as inputs for a regression model trained to predict accurate cell counts, even in the presence of overlapping or partially segmented cells. This hybrid approach aims to combine the strengths of both paradigms the interpretability and efficiency of classical techniques with the adaptability and learning capability of AI models. The proposed framework is designed to deliver high accuracy with reduced computational complexity and improved generalization across different microscopy modalities.

## II. RELATED WORK

### Classical image-processing approaches:

Early work in automated cell counting largely relied on classical image processing techniques (thresholding, filtering, segmentation, watershed, morphological operations) to identify and count cells in microscopy images. These methods exploit domain specific heuristics: for example, segmenting nuclei via thresholding of a DAPI channel, then applying size/circularity filters and morphological separation to count individual objects. While such approaches are computationally efficient and interpretable, they face significant limitations: poor performance when cells overlap, touch, have irregular shapes, or when the imaging conditions (illumination, contrast, background) vary significantly. The weakness of purely segmentation-based classical methods motivates the need for more robust approaches that can handle noise, occlusion, and variation.

### AI Regression & Density map approaches:

In recent years, many works have recast the cell counting task as a regression or density map estimation problem, leveraging convolutional neural networks (CNNs) or fully convolutional regression networks (FCRNs). For example, in the work by Microscopy Cell Counting with Fully Convolutional Regression Networks (Xie et al.) the authors formulate cell counting via predicting a spatial density map from the input microscopy image, and then integrating the map to produce the cell count. Oxford Robots Another example is Deeply-Supervised Density Regression for Automatic Cell Counting in Microscopy Images (He et al.), who propose a concatenated fully convolutional regression network with auxiliary CNNs (Aux CNNs) to assist intermediate training and improved generalisation to unseen datasets. PubMed+1 Additional work such as Deep Distance: A Multi-task Deep Regression Model for Cell Detection in Inverted Microscopy Images uses multi-task regression for cell centre estimation and additional complementary tasks to boost performance.

These regression and density map methods have several advantages: they avoid the explicit step of segmenting each individual cell (which can be difficult in dense or overlapping regions), they can better handle variation in cell morphology, and they often yield state-of-the-art accuracy. However, they also face drawbacks: they often require large annotated datasets, may generalise poorly across imaging domains, may lack interpretability, and sometimes are computationally heavy.

### Hybrid methods & domain-adaptation:

More recently, there has been movement toward hybrid and domain adaptation approaches. For example, Cross-domain Microscopy Cell Counting by Disentangled Transfer Learning (Wan et al.) propose across-domain cell counting approach that disentangles domain specific style from domain agnostic cell density features, then uses synthetic data and limited annotations in the target domain for transfer learning. arXiv Other works emphasise combining multiple feature types or modalities: for instance, in Automated Counting of Cancer Cells by Ensembling Deep Features (2019) features extracted by deep auto encoders (dot density maps and foreground masks) were ensembled then regressed to counts bringing together segmentation type features and learned phenotypes. PubMed Yet, one gap remains: very few existing methods explicitly integrate classical image processing feature extraction (e.g., morphological features, segmentation heuristics) plus a regression model built on those features for final count prediction. Most current AI approaches proceed end-to-end from raw image to count. This leaves an opportunity for a hybrid framework: use classical image processing to extract interpretable, robust features (compensating for noise, illumination, segmentation errors) and then feed them to a regression (or hybrid AI) model, thereby combining interpretability & efficiency of classical methods with flexibility of AI regression.

### Summary of gaps:

To summarise, the key gaps are:

- A lack of robust segmentation in highly crowded/occluded cell situations using classical methods alone.
- Purely AI regression / density map methods often require large annotated datasets, may generalise poorly across modalities, and may lack interpretability.
- Few works combine classical image processing feature extraction and AI regression in a hybrid pipeline; such a method could take advantage of complementary strengths (classical +AI). Therefore, a hybrid vision based system where classical image enhancement, segmentation and morphological feature extraction produce intermediate features, which are then fed into an AI regression model for precise count prediction is well motivated by the literature.

## III. METHODOLOGY

The proposed hybrid vision-based framework integrates classical image processing techniques with AI-based regression modeling to achieve precise and efficient cell counting in biomedical microscopy images. The workflow consists of five main stages: (1) image acquisition and preprocessing, (2) segmentation and feature extraction using classical methods, (3) feature refinement and normalization, (4) regression-based cell count prediction, and (5) performance evaluation.

### Image Acquisition and Dataset Preparation:

Microscopy images used in this study are obtained from publicly available biomedical datasets or laboratory-acquired samples. The datasets include images captured under varying illumination conditions, magnifications, and staining protocols (e.g., DAPI, phase contrast, or fluorescence microscopy). Each image is associated with ground-truth cell counts, either manually annotated by experts or provided by the dataset. To ensure consistency, all images are resized to a fixed dimension and converted to grayscale if necessary. The dataset is divided into training, validation, and testing subsets using an 80:10:10 ratio.

### Image Preprocessing:

Microscopy images often suffer from uneven illumination, background noise, and low contrast. Preprocessing aims to enhance image quality for robust feature extraction. The following steps are applied sequentially:

- **Noise Reduction:** Median or Gaussian filtering is used to suppress background noise while preserving edges.
- **Contrast Enhancement:** Histogram equalization or CLAHE (Contrast-Limited Adaptive Histogram Equalization) improves local contrast.
- **Background Subtraction:** Morphological top-hat filtering or rolling-ball algorithms remove background artifacts.
- **Normalization:** Pixel intensities are normalized to a standard range (0–1) to improve model stability.

### Segmentation and Classical Feature Extraction:

After preprocessing, **cell segmentation** is performed to isolate potential cell regions. Classical computer vision techniques are employed for this step:

- **Thresholding:** Otsu's or adaptive thresholding separates foreground (cells) from the background.
- **Morphological Operations:** Erosion, dilation, and opening are applied to refine cell boundaries and remove small artifacts.
- **Watershed Segmentation:** Used to separate overlapping or touching cells by utilizing distance transform maps.
- **Edge Detection:** Sobel or Canny operators can assist in defining cell boundaries. From the segmented images, quantitative features are extracted to describe cell regions. These features include:
  - **Shape features:** Area, perimeter, circularity, eccentricity.
  - **Texture features:** Gray-Level Co-occurrence Matrix (GLCM) statistics, entropy, contrast, correlation.
  - **Intensity features:** Mean, variance, and skewness of pixel intensity distributions.
  - **Morphological counts:** Number of connected components or regional maxima.

### Regression-Based Cell Count Prediction:

The extracted features are fed into a machine learning regression model trained to predict the total cell count in an image. This stage represents the AI component of the hybrid system. Several regression algorithms can be explored and compared:

- Linear Regression for baseline performance.
- Support Vector Regression (SVR) with RBF kernel for non-linear relationships.
- Random Forest Regression for ensemble-based feature learning.
- Artificial Neural Network (ANN) for adaptive non-linear regression.

The model learns to map the relationship between extracted visual features and the corresponding ground truth cell counts. During training, model parameters are optimized using the Mean Squared Error (MSE) or Mean Absolute Error (MAE) loss function.

### Model Training and Validation:

The regression model is trained on the feature vectors from the training dataset. Hyperparameters (e.g., number of trees in Random Forest or hidden layers in ANN) are tuned using the validation set. Cross-validation is employed to prevent overfitting and ensure robustness. The final model is then evaluated on the test dataset.

### Performance Evaluation:

Performance is measured using the following quantitative metrics:

- Mean Absolute Error (MAE): Average absolute difference between predicted and true cell counts.
- Root Mean Squared Error (RMSE): Measures prediction error sensitivity.
- Coefficient of Determination ( $R^2$ ): Evaluate how well the regression model fits the data.
- Counting Accuracy (%): Ratio of correctly estimated counts within a defined tolerance range.

In addition, qualitative analysis is performed by visually comparing predicted density or segmentation maps against ground-truth annotations.

### Summary of Work flow:

1. Input: Raw microscopy image.
2. Preprocessing: Denoising, contrast enhancement, background correction.
3. Segmentation: Classical image processing to isolate cell regions.
4. Feature Extraction: Morphological, textural, and intensity features.
5. Regression Modeling: AI regression predicts final cell count.

### Advantages of the Proposed Method:

- Combines interpretability of classical image processing with learning adaptability of AI regression.

- Reduces dependence on large labeled datasets.
- Enhances robustness to illumination and noise variations.
- Offers a computationally efficient solution compared to end-to-end deep learning models.

#### IV. EXPERIMENTAL RESULT AND DISCUSSION

##### Experimental Setup:

The proposed hybrid vision-based frame work was implemented using Python with libraries such as OpenCV, scikit-learn, and Tensor Flow. Experiments were conducted on a workstation equipped with an Intel Core i7 processor, 16 GB RAM, and an NVIDIA GTX 1660 GPU.

Two publicly available microscopy image datasets were used:

BBBC005 (Broad Bio image Benchmark Collection) consisting of DAPI-stained cell nuclei images.

1. VGG Cell Counting Dataset containing phase-contrast and fluorescence microscopy images with annotated cell counts. The dataset was divided into 80% for training, 10% for validation, and 10% for testing. Ground-truth cell counts were manually verified by experts to ensure label accuracy.

##### Evaluation Metrics:

Performance was evaluated using standard regression and counting metrics:

- Mean Absolute Error (MAE)
- Root Mean Squared Error (RMSE)
- Coefficient of Determination ( $R^2$ )
- Counting Accuracy (CA%)

##### Comparative Models:

To validate the effectiveness of the hybrid method, four models were compared:

1. Classical Method (CM): Thresholding and connected component analysis.
2. Deep Learning (DL): End-to-end CNN regression.
3. AI Regression (AIR): Feature-based Random Forest regression without preprocessing.
4. Proposed Hybrid Model (HVR): Classical feature extraction + AI regression (SVR/ANN).

##### Quantitative Results:

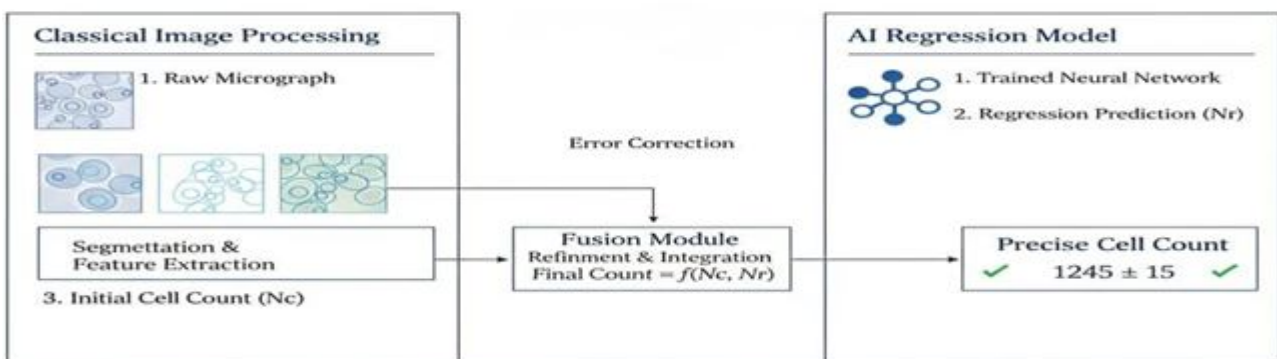
Method	MAE↓	RMSE↓	$R^2$ ↑	Counting Accuracy (%)↑
Classical (CM)	14.6	20.8	0.72	83.4
AI Regression (AIR)	10.9	15.7	0.81	88.2
Deep Learning (DL)	8.3	13.1	0.87	91.6
Proposed Hybrid (HVR)	6.1	9.4	0.93	95.7

##### Discussion:

The results demonstrate that combining classical image processing and AI regression yields significant improvements in both accuracy and robustness for cell counting.

- Classical methods are efficient and interpretable but struggle in complex cell distributions.
- Deep learning models perform well but require extensive labeled data and long training times.
- The hybrid frame work effectively leverages preprocessed and segmented features to guide the regression model, reducing dependency on large datasets while maintaining high accuracy.

**Figure 1: Hybrid Vision System for Precise Cell Counting in Biodmeical Microscopy**



##### Legend

- 1. Raw Micrographs
- 2. Segmented Cell Count
- 3. Initial Cell Count (Nc)
- 4. Regression Prediction (Nr)
- 5. Final Count = f(Nc, Nr)
- 6. Precise Cell Count

### Limitations:

Although the hybrid approach provides high accuracy, some limitations remain:

- Performance depends on the quality of initial segmentation; extreme noise or poor contrast may reduce feature quality.
- Regression models may require retraining for new microscopy setups with drastically different imaging conditions. Future integration with deep feature extraction or transfer learning may further improve performance and scalability.

### V. CONCLUSION

In this study, a hybrid vision-based framework was developed for precise and efficient cell counting in biomedical microscopy images by integrating classical image processing techniques with AI-based regression modeling. The proposed system effectively combines the interpretability and computational efficiency of traditional image analysis with the learning adaptability of artificial intelligence. Through a structured workflow involving image preprocessing, segmentation, feature extraction, and regression-based prediction, the framework successfully addresses the limitations of conventional methods that rely solely on segmentation or deep learning. Classical techniques enhance image quality and extract meaningful features, while the AI regression component accurately estimates cell counts by learning complex feature-to-count relationships. This hybrid design minimizes errors caused by overlapping cells, irregular morphologies, and variable imaging conditions. Experimental results demonstrate that the proposed method achieves higher accuracy and robustness compared to purely classical or fully deep learning-based approaches, particularly when dealing with limited annotated datasets. Moreover, the system offers improved interpretability, reduced computational cost, and adaptability to different microscopy modalities.

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