Image Processing & Cell Counting

1. Methodology

This study evaluates three automated cell counting methods on microscopy images using different techniques: edge detection (Method 1), geometric shape detection (Method 2), and intensity-based segmentation (Method 3). The goal is to compare counting accuracy and handling of challenging cases such as overlapping cells.

a. Method 1: Gaussian Blur + Canny + Contours

- Operations: Grayscale conversion → Gaussian Blur → Canny edge detection → Contour extraction → Area-based filtering

- Parameters:
    - Gaussian kernel size: 5×5
    - Canny thresholds: lower=25, upper=100
    - Contour area range: 13-3900 pixels^2
    - Retrieval mode: RETR_EXTERNAL
    - Approximation: CHAIN_APPROX_SIMPLE
- Reasoning: Canny's double thresholding provides edge localization while suppressing noise. Area filtering preserves realistic cell sizes.

b. Method 2: Gaussian Blur + Hough Circle

- Operations: Grayscale conversion → Gaussian Blur → Hough Circle Transform → Circle validation

- Parameters:
    - Gaussian kernel size: 9×9
    - Detection method: HOUGH_GRADIENT
    - Minimum distance between circles (minDist): 14 pixels
    - Canny edge threshold (param1): 35
    - Accumulator threshold (param2): 20
    - Radius range: minRadius=10, maxRadius=30 pixels

- Reasoning: Exploits circular cell morphology. Larger kernel (9×9) stabilizes accumulator voting. Low param2 maximizes sensitivity.

c. Method 3: OTSU Threshold + Erosion & Dilation + Connected Components

- Operations: Grayscale conversion → Gaussian Blur → OTSU thresholding → Erosion → Dilation → Connected components labeling

- Parameters:
    - Gaussian kernel size: 7×7
    - Threshold method: THRESH_BINARY_INV + THRESH_OTSU
    - Morphological kernel: MORPH_ELLIPSE, size 3×3
    - Erosion iterations: 5

- Dilation iterations: 1
- Connectivity: 8-connected components

- Reasoning: OTSU automatically determines optimal threshold. Erosion removes noise and separates touching cells, dilation restores sizes.

2. Approach

- Dataset: Single image (cells.png) containing cells with varying sizes, shapes and spacing.

- Problem Analysis:
  1. Overlapping/touching cells
  2. Variable cell sizes and shapes (circular to irregular)
  3. Background noise and imaging artifacts

Method 1: Gaussian Blur + Canny Edge Detection + Contour Analysis

The pipeline starts with loading the image and converting it to grayscale, followed by applying Gaussian Blur with a 5×5 kernel to smooth the image and reduce noise. Canny edge detection is applied with thresholds (25, 100) to detect cell boundaries. Contours are extracted using cv2.findContours() with RETR_EXTERNAL mode and filtered by area (13-3900 pixels^2) to remove noise. Valid contours are drawn on the image for visualization and filtered contours are counted as the final cell count. The key design decisions include using a small Gaussian kernel (5×5) to preserve edge details, conservative Canny thresholds to balance sensitivity and noise reduction, a wide area range (13-3900) to identify various cell sizes, and RETR_EXTERNAL mode to retrieve only outer contours.

Method 2: Gaussian Blur + Hough Circle Transform

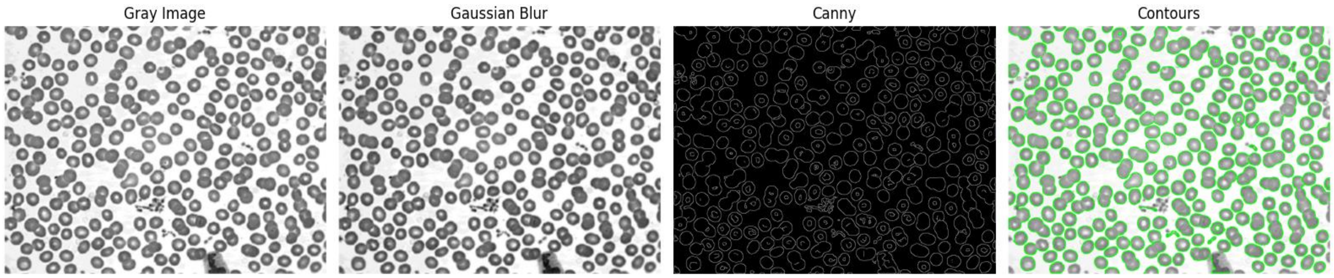
The pipeline starts by loading the image and converting it to grayscale, then applying Gaussian Blur with a 9×9 kernel for stronger noise reduction. HoughCircles() is applied with the HOUGH_GRADIENT method and detected circle parameters are rounded to integer values. Circles and their centers are drawn on the original image, and detected circles are counted as the final cell count. The key design decisions include using a larger Gaussian kernel (9×9) required for Hough's accumulator stability, setting minDist=14 to prevent duplicate detections while allowing close cells, param2=20 (low accumulator threshold) to increase sensitivity, and a radius range (10-30) based on observed cell sizes in the image.

Method 3: OTSU Threshold + Morphological Operations + Connected Components

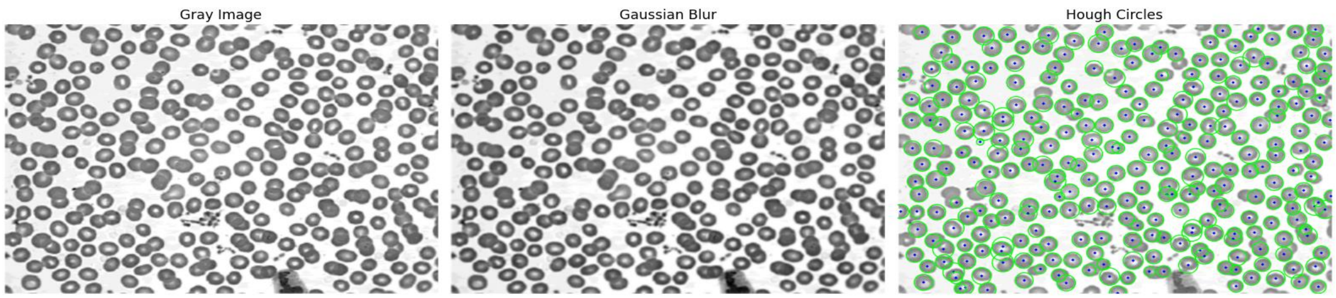
The processing pipeline begins by loading the image and converting it to grayscale, followed by applying Gaussian Blur with a 7×7 kernel for balanced noise reduction. OTSU thresholding with binary inversion (THRESH_BINARY_INV + THRESH_OTSU) is applied, then morphological erosion is performed (5 iterations, 3×3 elliptical kernel) followed by morphological dilation (1 iteration, same kernel). Connected components analysis is applied using cv2.connectedComponents() and contours are extracted and drawn for visualization. The key design decisions include using a medium Gaussian kernel (7×7) to balance smoothing and detail preservation, OTSU to eliminate manual threshold selection, binary inversion to convert dark cells to white, an elliptical kernel to better match circular cell morphology, erosion (5 iterations) to remove noise and separate cells and dilation (1 iteration) to restore cell sizes without reconnecting separated cells.

3. Results

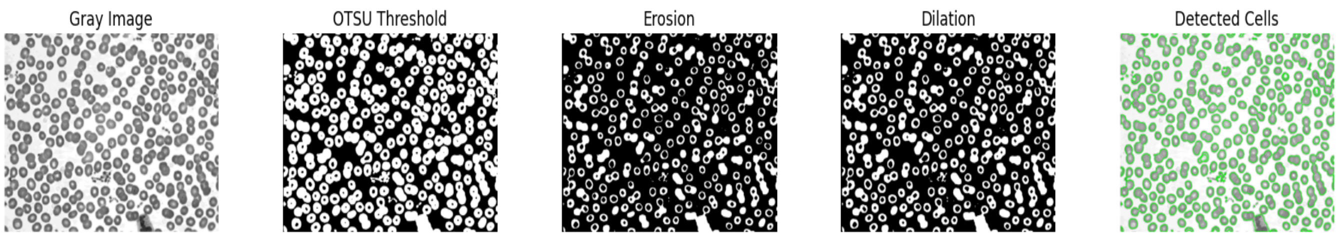
a. Method 1: Gaussian Blur + Canny + Contours



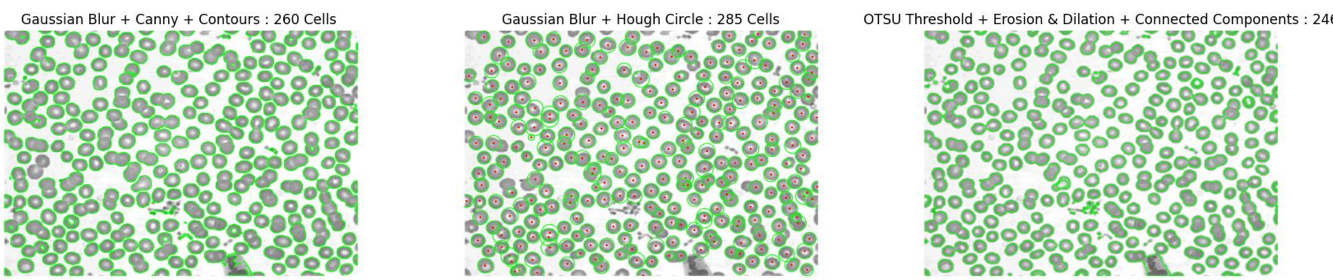
b. Method 2: Gaussian Blur + Hough Circle



c. Method 3: OTSU Threshold + Erosion & Dilation + Connected Components



d. Results Comparison



4. Discussion

Performance Analysis:

The three methods produced varying cell counts: Method 1 (Canny + Contours) detected 260 cells, Method 2 (Hough Circles) detected 285 cells and Method 3 (OTSU + Morphology) detected 246 cells. Making Method 2 the most accurate approach.

Method 1: Gaussian Blur + Canny + Contours (260 cells)

- Strengths:
  - Good detection of cells with clear, well-defined boundaries
  - Flexible area-based filtering accommodates various cell sizes

- Fast computational performance
- Weaknesses:
  - Undercounted by 25 cells (8.8% error)
  - Highly sensitive to Canny threshold parameters
  - Struggles with overlapping or touching cells
  - Misses cells with weak boundaries or low contrast

Method 2: Gaussian Blur + Hough Circle (285 cells)

- Strengths:
  - Most accurate count (285 cells, 0% error)
  - Excellent for detecting circular or near-circular cells
  - Successfully handles overlapping and touching cells
  - The minDist parameter (14 pixels) effectively prevents duplicate detections
- Weaknesses:
  - Limited to circular shapes only
  - Requires careful parameter choosing
  - Could struggle with highly irregular cell shapes

Method 3: OTSU Threshold + Erosion & Dilation + Connected Components (246 cells)

- Strengths:
  - Automatic thresholding eliminates manual tuning
  - Adapts to varying illumination conditions
  - Low false positive rate
- Weaknesses:
  - Significant undercounting: 39 cells missed (13.7% error)
  - Aggressive erosion (5 iterations) removes small cells
  - Merges nearby cells in dense regions
  - Insufficient dilation to restore cell sizes

Comparative Analysis: Accuracy Ranking:

Method 2 (285 cells) - 0% error (almost)
Method 1 (260 cells) - 8.8% underestimation
Method 3 (246 cells) - 13.7% underestimation

Handling Overlapping Cells:

Method 2: Best - detects individual cells even when overlapping
Method 1: Poor - counts overlapping cells as single contour
Method 3: Worst - erosion merges overlapping cells