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Hybrid Deep Learning–Based Computer Vision Framework for Brain Organoid and Embryoid Body Segmentation

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Abstract

Image processing plays a crucial role in enhancing the quality of biological images, enabling accurate analysis and interpretation. In this work, brain organoid images from a Kaggle dataset were pre-processed and analysed using Gaussian, Median, Histogram Equalization, Canny, Sobel, Otsu, and Watershed segmentation techniques. Median filtering demonstrated superior performance in noise removal and edge preservation compared to Gaussian filtering. Histogram equalization effectively improved contrast, while advanced edge-detection and segmentation methods provided deeper structural insights. Finally, a hybrid approach combining Median Filtering, Histogram Equalization, and the Watershed Algorithm along with deep learning models produced the most reliable segmentation results, offering clearer boundaries and improved feature extraction for organoid analysis.

Key Words

Canny Edge Detection, Gaussian Filter, Histogram Equalization, Image Processing, Median Filter, Otsu Thresholding, Segmentation, Sobel Operator, Stem Cell Imaging, Watershed Algorithm

Introduction

Computer vision automates the detection, classification, and segmentation of complex image patterns. It reduces manual effort and improves the accuracy and speed of biological and medical image analysis. Computer vision enables automated analysis of microscopic structures such as organoids, stem cells, and tissues. It supports advanced diagnostics and accelerates biological discoveries through automated segmentation and quantification. Image processing improves the quality, clarity, and interpretability of biological images for accurate analysis. It enables efficient feature extraction, noise removal, and segmentation essential for scientific and medical applications. Stem cells are undifferentiated cells capable of developing into specialized cell types, essential for growth and regeneration. A real-life example is the use of induced pluripotent stem cells (iPSCs) to grow organoids for studying neurological disorders such as Alzheimer’s disease.

Methodology

Image Preprocessing

Brain organoid images collected from a Kaggle dataset. Used for noise reduction, contrast enhancement, and segmentation analysis.

Gaussian Filter

Gaussian filter is mainly used to smoothen brain and embryo images by averaging nearby pixels using the following formula.

$$\text{Gaussian Function (2D)} = G(x,y) = \frac{1}{2\pi\sigma^2}\, e^{-\frac{x^2+y^2}{2\sigma^2}} \qquad (1)$$

Where:

x, y= distance from the center pixel

σ= standard deviation

e= exponential function

It reduces noise and blurs small variations while preserving the overall structure, improving clarity for further analysis shown in Figure 1. The process converts the organoid image to the correct color format, applies smoothing, and displays the original and filtered images side by side. Pseudocode 1 converts a brain organoid image from BGR to RGB format and applies a Gaussian filter to smooth the image.

Pseudocode 1: Applying Gaussian Filter to the Brain and Embryoid Bodies

BEGIN

Input the brain organoid image.

Convert the input image from BGR color format to RGB format.

Apply Gaussian Blur with a 5×5 kernel to smooth the image.

Create a figure window containing two subplots.

Display the original RGB image in the first subplot.

Display the Gaussian-filtered image in the second subplot.

Show the final output plot.

END

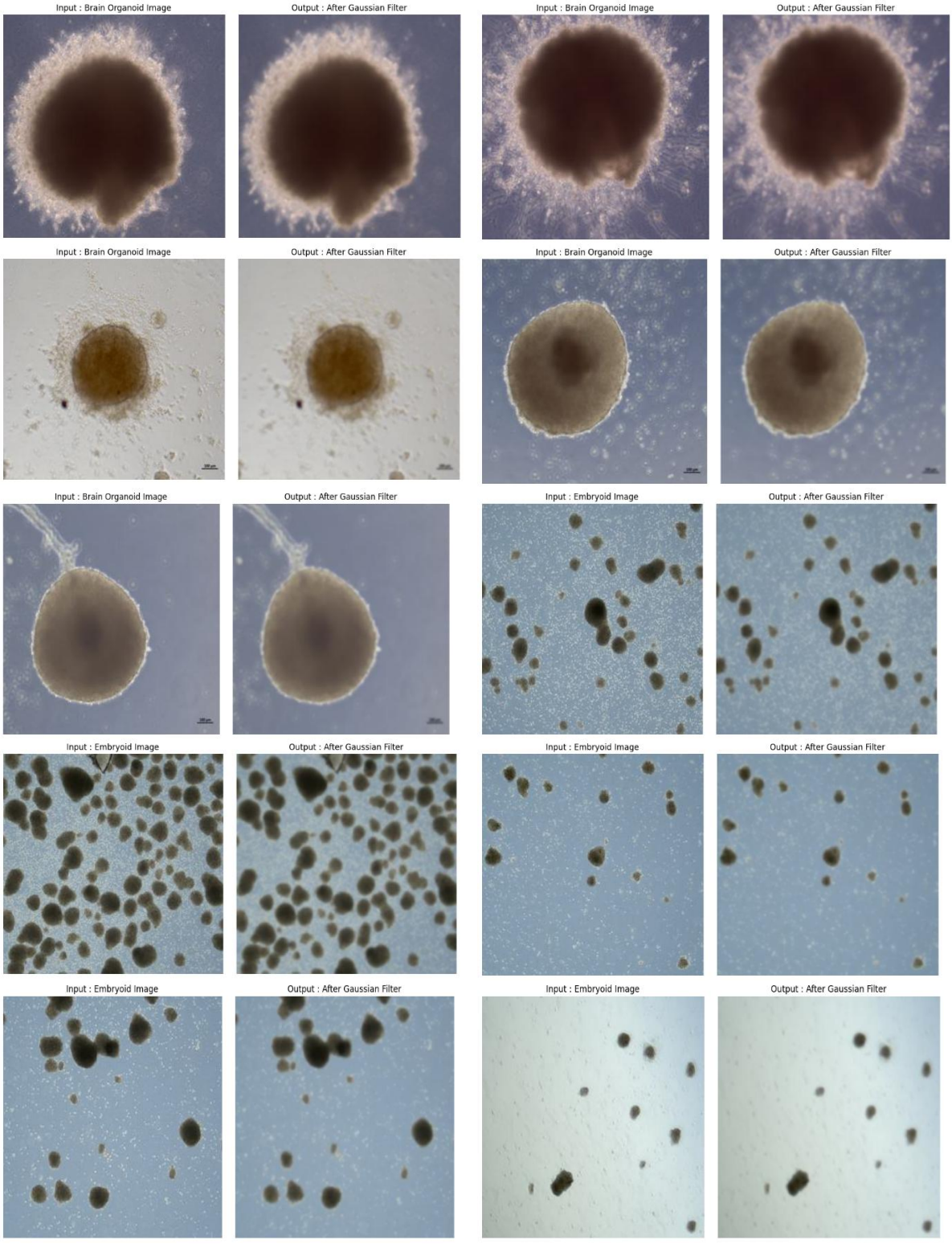


Figure 1: Gaussian Filtered Image for Enhanced Organoid Visualization

Median Filter

Median filter is a preprocessing technique mainly used to remove salt-and-pepper noise from images. Since the images are taken from microscopic equipment, they often contain such noise. The filter works by replacing each pixel with the median value of its surrounding kernel instead

of averaging, which helps preserve important edges in organoid imagery shown in Figure 2. The following formula is used.

$$I_{\text{filtered}}(x, y) = \text{median}\{ I(i, j) \mid (i, j) \in N(x, y) \} \qquad (2)$$

Where:

$I(x, y)$= original image pixel

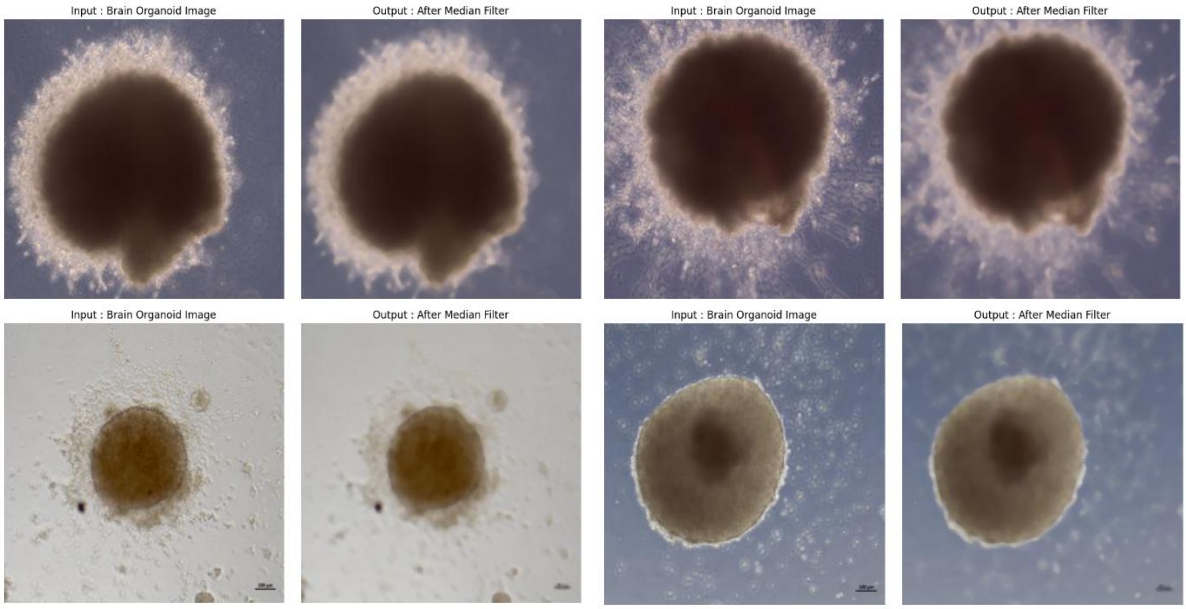
$I_{\text{filtered}}(x, y)$= output pixel value

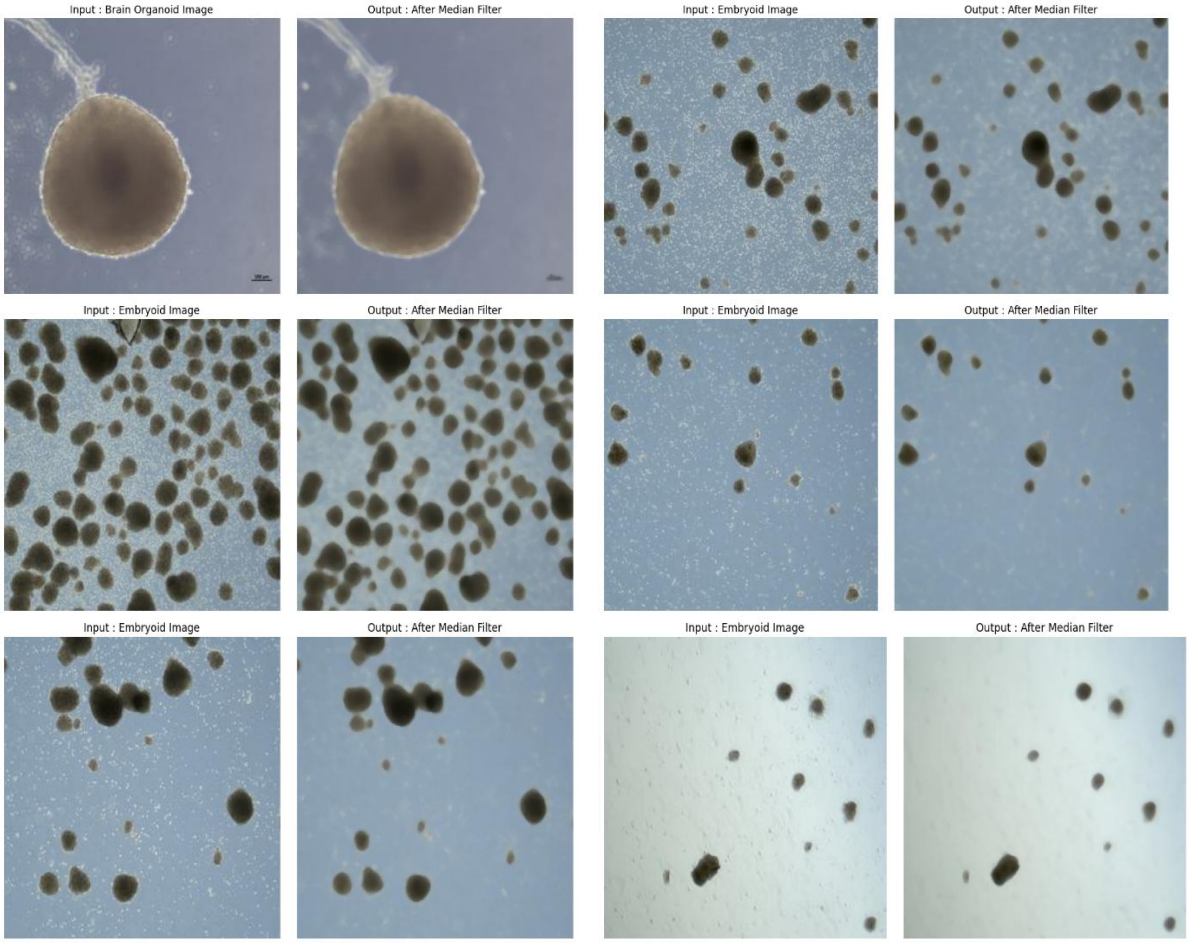
$N(x, y)$= neighbourhood window around (x, y)

median = the middle value when all values in the window are sorted

Pseudocode 2: Applying Median Filter to the Brain and Embryoid Bodies

```
BEGIN
  Define a list of brain organoid image paths.
  FOR each image path in the list:
     Load the image from the given path.
     IF the image is not loaded correctly:
        Display an error message and continue to the next image.
     Convert the loaded image from BGR format to RGB format.
     Apply a Median Filter with kernel size 5 to the RGB image.
     Create a figure window with two subplots.
     Display the original RGB image in the first subplot.
     Display the median-filtered image in the second subplot.
     Show the plotted output.
  END FOR
END
```




Input : Brain Organoid Image
Output : After Median Filter
Input : Embryoid Image
Output : After Median Filter
Input : Embryoid Image
Output : After Median Filter
Input : Embryoid Image
Output : After Median Filter
Input : Embryoid Image
Output : After Median Filter
Input : Embryoid Image
Output : After Median Filter

c

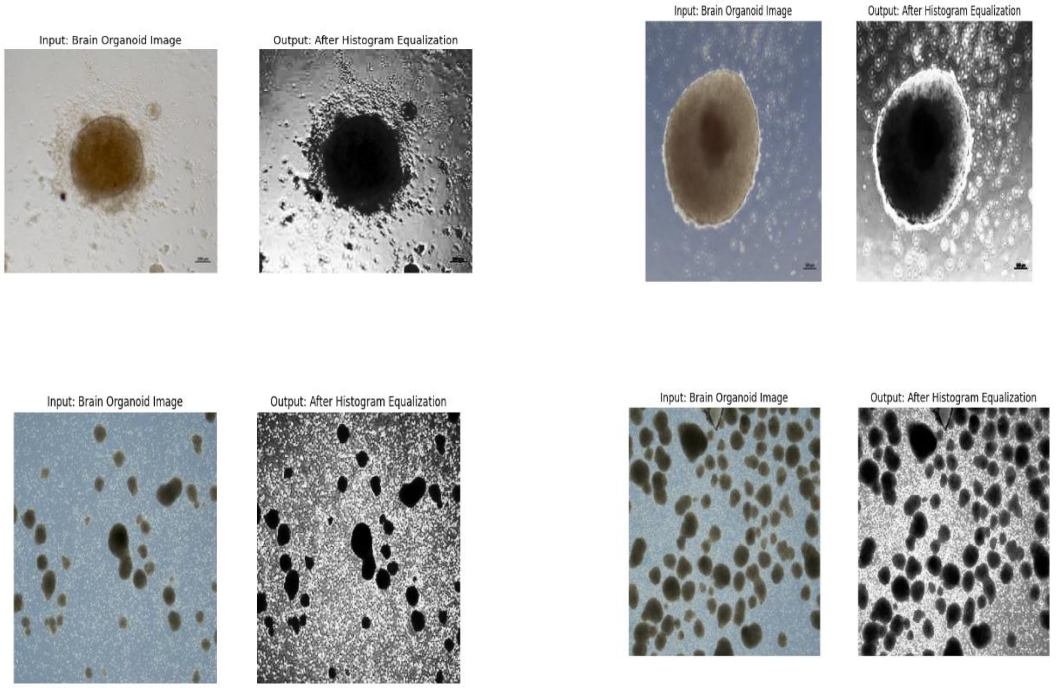

Input: Brain Organoid Image
Output: After Histogram Equalization
Input: Brain Organoid Image
Output: After Histogram Equalization
Input: Brain Organoid Image
Output: After Histogram Equalization
Input: Brain Organoid Image
Output: After Histogram Equalization

Figure 3: Histogram Equalized Image for Enhanced Organoid Visualization

Segmentation

Otsu's method is an automatic global thresholding technique that finds the threshold value that minimizes intra-class variance between foreground and background.

Otsu selects threshold **t** that minimizes:

$$\sigma_w^2(t) = q_1(t)\sigma_1^2(t) + q_2(t)\sigma_2^2(t) \qquad (3)$$

Where:

q_1, q_2= class probabilities

σ_1^2, σ_2^2= class variances

Watershed Segmentation

Watershed treats the image as a topographic surface where bright areas are peaks and dark areas are valleys. Flooding the valleys helps detect object boundaries.

The segmentation is based on the **gradient magnitude**:

$$W = \text{watershed}(\nabla I) \qquad (4)$$

Where:

∇I= Gradient of the image.

Canny Edge Detection

Canny is a multi-stage edge detection algorithm that finds sharp, thin, continuous edges based on gradients.

Gradient magnitude:

$$G = \sqrt{G_x^2 + G_y^2} \qquad (5)$$

Direction:

$$\theta = \tan^{-1}\left(\frac{G_y}{G_x}\right) \qquad (6)$$

Sobel Edge Detection

Sobel uses convolution kernels to detect changes in intensity (edges) in horizontal and vertical directions.

$$G_x = I * S_x, G_y = I * S_y \quad (7)$$

$$G = \sqrt{G_x^2 + G_y^2} \quad (8)$$

The figure 4 illustrates how different image processing techniques such as Otsu, Watershed, Canny, and Sobel extract distinct structural and boundary features from the original organoid image. Each method highlights specific details, enabling clearer visualization and improved morphological analysis.

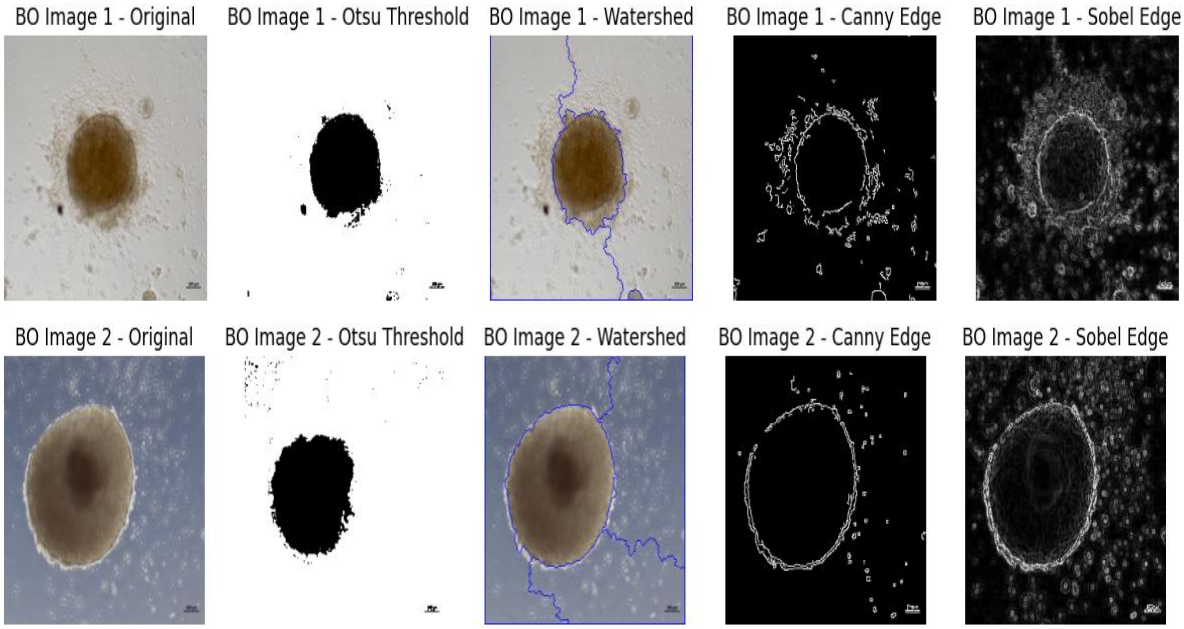


Figure 4: Original vs. Otsu, Watershed, Canny, and Sobel Image Processing Results.

Proposed Hybrid Model:

In this work, the Watershed algorithm is implemented for accurate segmentation of brain organoid cells, as it effectively separates touching or overlapping cell regions. To improve the segmentation accuracy, a hybrid preprocessing pipeline is designed using three essential steps:

- Median Filtering – removes salt-and-pepper noise while preserving fine cell boundaries.
- Histogram Equalization – enhances contrast to make cell edges more distinguishable.
- Watershed Segmentation – identifies and separates individual cell regions with high precision.

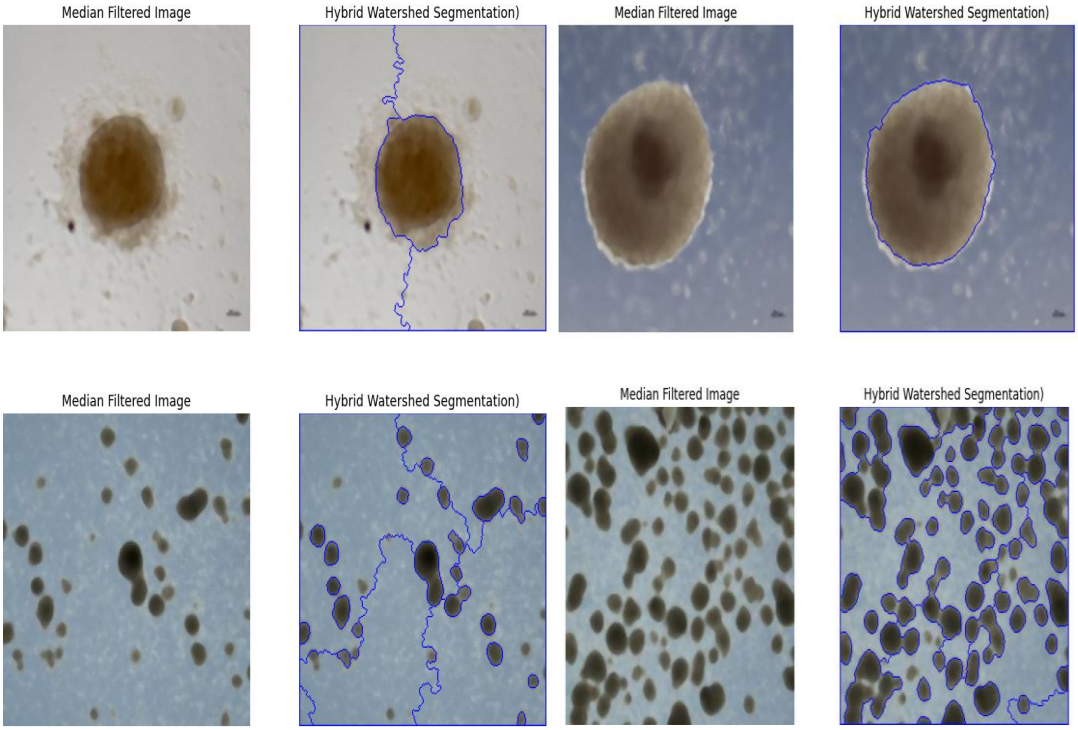


Figure 5: Output of the Proposed Hybrid Pipeline

This hybrid model is crucial because organoid images often contain noise, low contrast, and overlapping cell structures, which make direct segmentation difficult. By combining noise reduction, contrast enhancement, and a strong boundary-based segmentation method, the proposed approach improves clarity, preserves cellular details, and enables more accurate morphological and quantitative analysis of stem cell–derived organoids shown in Figure 5.

Hybrid Ensemble Model (CNN + DenseNet + Voted Fusion)

Soft-voting ensemble formula combining CNN and DenseNet probabilities. The ensemble integrates CNN's strong texture learning with DenseNet's deep structural feature extraction. Soft-voting averages both model outputs, reducing classification noise and boundary errors. The final ensemble achieves the highest accuracy and most stable predictions for organoid analysis shown in Table 3.

$$P_{final} = \frac{P_{CNN} + P_{DenseNet}}{2} \qquad (9)$$

Final class is:

$$\hat{y} = \arg\max(P_{final}) \qquad (10)$$

Result Analysis

Gaussian filter provides smooth noise reduction and is effective for removing random sensor noise in microscopic organoid images. Median filter preserves edges and removes salt-and-pepper noise more efficiently without distorting organoid boundaries. Choosing the best filter

depends on whether smoothness (Gaussian) or edge clarity (Median) is more important for the analysis shown in Table 1.

Table 1: Comparison of Gaussian Filter and Median Filter

Criteria	***Gaussian Filter***	***Median Filter***	***Best For***
Type of Noise Removed	Random/Gaussian noise	Salt-and-pepper noise	Depends on noise present
Smoothing Quality	Produces smooth, soft images	Moderate smoothing	Gaussian
Edge Preservation	Slightly blurs edges	Strong edge preservation	Median
Effect on Organoid Boundaries	Can blur thin boundaries slightly	Preserves boundaries clearly	Median
Image Texture	Natural, continuous smoothing	May slightly distort smooth textures	Gaussian
Kernel Operation	Weighted averaging	Median replacement	—
Speed	Faster	Slightly slower	Gaussian
Suitability for Microscopy Images	Excellent for reducing sensor noise and uneven illumination	Excellent for removing impulse noise	Depends on noise type
Preprocessing for Segmentation	Produces clean, smooth masks	Ensures sharp edges for accurate boundary detection	Median (if boundaries matter most)

Table 2 compares Otsu Thresholding, Watershed, Canny, and Sobel methods based on working principle, strengths, weaknesses, and output quality for stem-cell organoid segmentation. Watershed segmentation achieves the most accurate morphological boundary extraction compared to the other methods. Watershed segmentation provides the clearest and most continuous organoid boundary, making it ideal for morphological analysis. Otsu Thresholding fails to preserve shape details, while Canny and Sobel produce fragmented or noisy edge maps. Thus, Watershed is the most reliable technique for accurate and complete organoid segmentation.

Table 2: Comparison of Image Segmentation and Edge-Detection Algorithms

Algorithm	***Working Principle***	***Strengths***	***Weaknesses***	***Result Quality***
Otsu Thresholding	Global threshold based on intensity variance	Fast, automatic	Fails if intensity is uneven; loses shape	Poor

Watershed Segmentation	Flooding based on gradients and markers	Best boundary detection, shape preserved	Can over-segment if markers are weak	**Best**
Canny Edge Detection	Multi-stage gradient edge detection	Sharp, continuous edges	Too many edges in noisy images	Moderate
Sobel Edge Detection	Gradient filter (X & Y)	Good for texture detection	Outputs noisy edge map	Low–Moderate

DenseNet performs better than the standalone CNN by capturing finer structural details, leading to higher accuracy and recall. The ensemble of CNN and DenseNet achieves the best overall performance by combining the strengths of both models for more stable and accurate organoid classification.

Table 3: Comparative Result Table

Method	**Accuracy**	**Precision**	**Recall**	**F1-Score**
CNN	0.96	0.95	0.97	0.96
DenseNet	0.98	0.97	0.99	0.98
Ensemble (CNN + DenseNet)	**0.989**	**0.985**	**0.99**	**0.985**

Conclusion

A hybrid segmentation model combining Median Filter + Histogram Equalization + Watershed was implemented. This approach reduces noise, enhances contrast, and produces highly accurate and separated organoid segments for analysis. This study systematically evaluated essential preprocessing and segmentation techniques for brain organoid imagery. The results show that the hybrid model—Median Filter + Histogram Equalization + Watershed—achieves the most accurate structural segmentation. This combined approach effectively reduces noise, enhances contrast, and produces precise region boundaries, making it highly suitable for biological image analysis and downstream computational tasks. Deep Learning is essential in modern computer vision because it automatically learns meaningful features directly from images without manual intervention. In biological and organoid imaging, where structures are highly complex, irregular, and vary in shape or intensity, deep neural networks capture subtle textures, boundaries, and cellular variations far better than traditional image processing techniques. Models like CNNs and DenseNet extract multi-level features—from edges to high-level morphological patterns—leading to superior segmentation, classification, and noise-robust interpretation. This makes deep learning the most powerful and accurate approach for analyzing microscopic, biomedical, and organoid image datasets.

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