Myelinn - Neural Biopsy Report

Introduction

The dataset for this project consisted of high-resolution `.png` images, each representing a single fossicle. These images contained structures of interest, including myelin sheaths and red blood cells (RBCs). Each image had a size of 4725×4822 pixels, offering detailed views of these structures. The aim was to develop an image processing pipeline to extract quantitative insights related to neurological health.

Objective

The primary goal of this project was to analyze neuro biopsy images to quantitatively evaluate key structural characteristics of myelin sheaths, which are critical for understanding neurological health and disorders. Specifically, the objectives included:

1. Diameter Calculation:

- Measure the diameters of myelin sheaths visible in the images.
- Insights into the size and uniformity of myelin sheaths provide crucial indicators of nerve fiber health.

2. Thickness Calculation:

- Estimate myelin sheath thickness by detecting outer and inner contours.
- Calculate the difference between the radii of the outer and inner boundaries.
- This metric indicates the structural integrity and functional capability of myelin sheaths.

3. Classification of Red Blood Cells (RBCs):

- Identify and classify RBCs based on distinct morphological features.
- Exclude RBCs from further processing to avoid interference with the analysis of myelin sheaths.

Data Description

- **Image Type:** High-resolution `.png`
- **Image Size:** 4725×4822 pixels

- **Content:** Neuro biopsy structures such as myelin sheaths and RBCs.

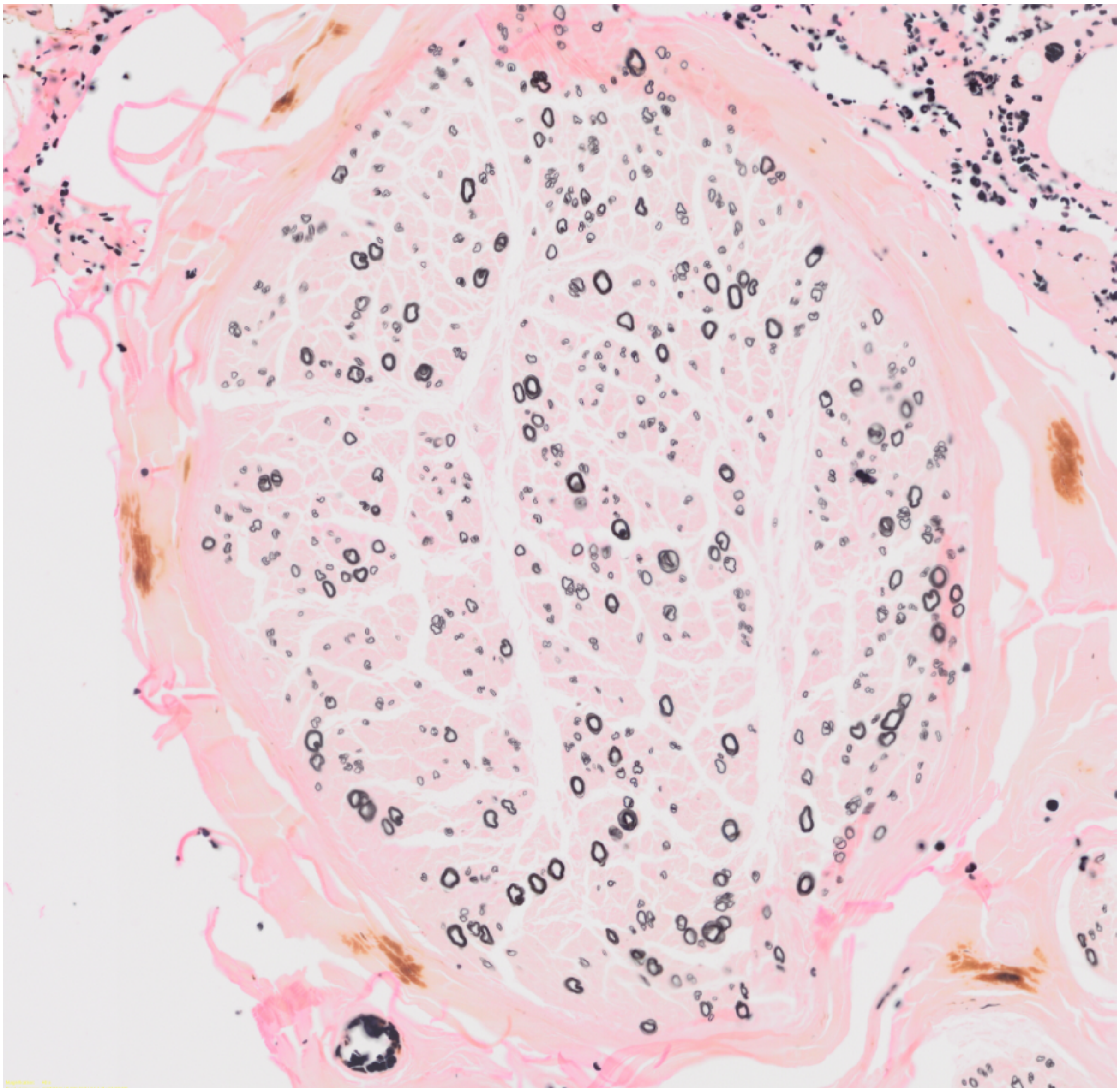


Fig-1: Original high-resolution neuro biopsy image representing a single fossicle. The structures of interest, such as myelin sheaths and red blood cells (RBCs), are clearly visible. This image serves as the starting point for preprocessing and analysis steps.

Preprocessing of Images

To ensure efficient and accurate analysis, preprocessing steps were implemented systematically:

1. Patch Extraction:

- **Process:** Extracted 512×512 patches from the original image using libraries like OpenCV and PIL.
- **Purpose:** Localized processing of smaller patches reduces computational overhead and ensures focused analysis.
- **Output:** Each patch was saved as a separate image file for further processing.

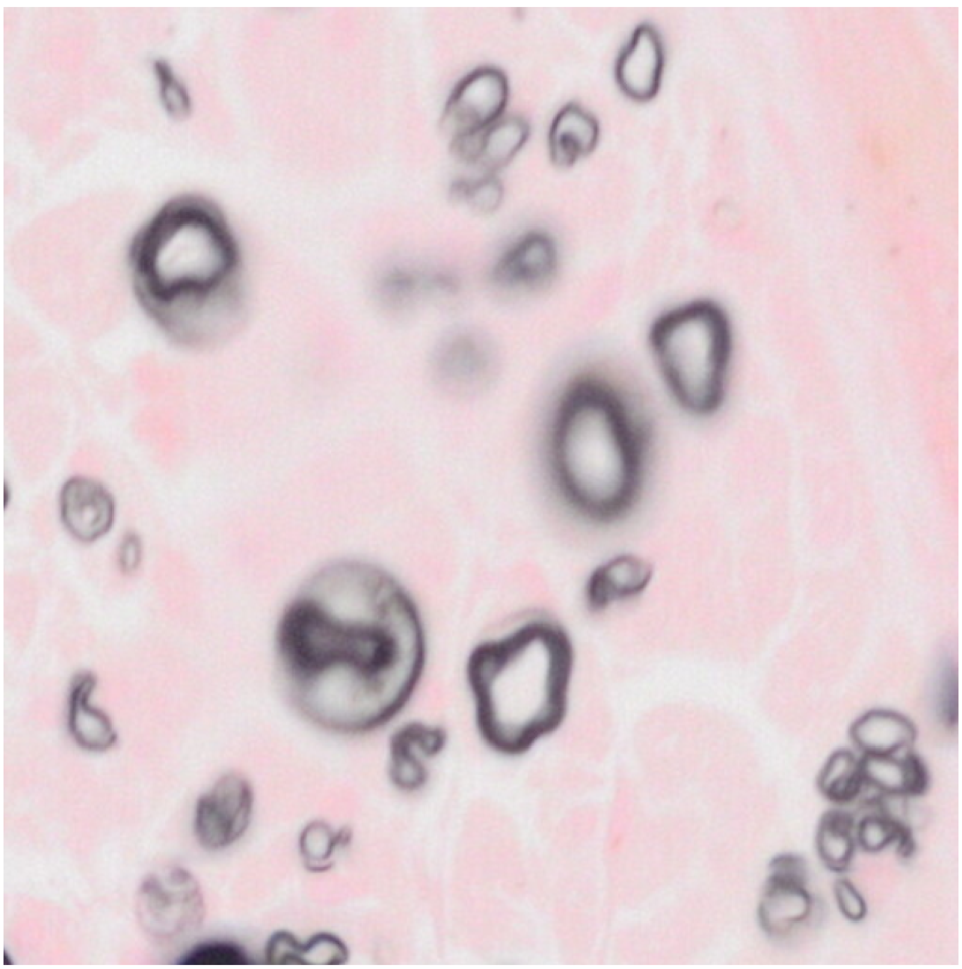


Fig-2: Extracted 512×512 patch from the original neuro biopsy image. This patch represents a localized region used for detailed analysis, allowing for efficient computational processing and focused segmentation of myelin sheaths and RBCs.

2. Morphological Operations:

a. Gaussian Blur:

- **Description:** A Gaussian Blur is applied to smooth the image and reduce noise. This technique uses a Gaussian function to create a kernel that blurs the image while preserving edges to a certain extent.
- **Example:** Applying a Gaussian Blur with a kernel size of (5×5) to the grayscale image ensures that subtle variations in intensity caused by noise are reduced.

b. Closing:

- **Description:** Morphological closing involves dilation followed by erosion. It is used to close small holes or gaps in object boundaries.
- **Example:** A 3×3 kernel is used to perform closing, ensuring that discontinuities in the myelin sheath boundaries are corrected for better contour detection.

c. Erosion:

- **Description:** Erosion reduces the size of objects by eroding their boundaries. It removes small noise but can slightly shrink object areas.
- **Example:** Using a 3×3 kernel, erosion removes tiny irrelevant artifacts from the image, keeping only the significant structures like myelin sheaths intact.

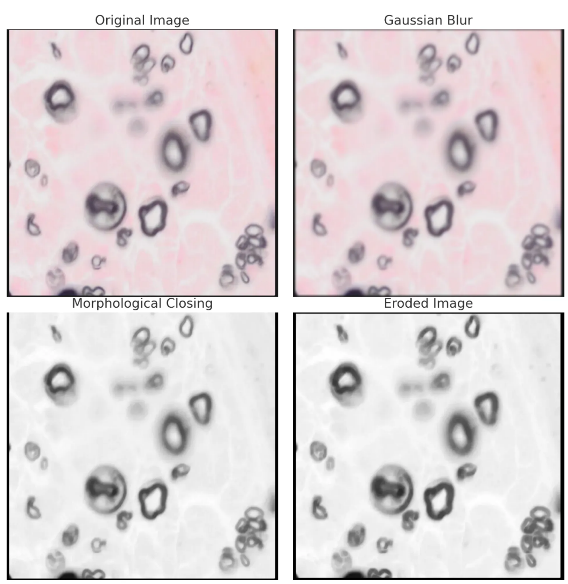
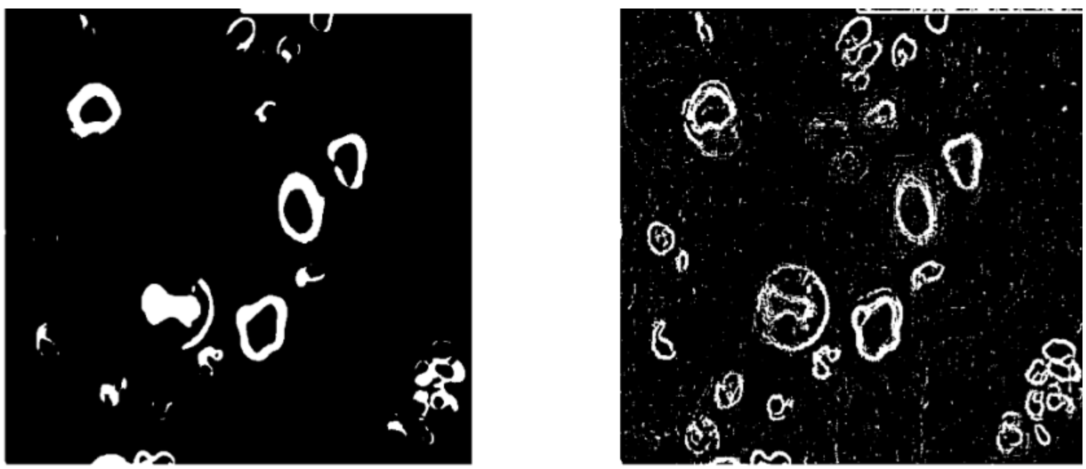


Fig-3: Visualization of preprocessing techniques: Original Image, Gaussian Blur, Morphological Closing, and Eroded Image.

3. Thresholding:

- **Global Thresholding:**
  - Initial experiments with global thresholding were unsatisfactory as it applied a uniform threshold, which sometimes eliminated critical details in certain areas of the image.
- **Adaptive Thresholding:**
  - Adaptive thresholding dynamically adjusts the threshold values based on local pixel intensity variations. It ensures that both bright and dark regions are segmented accurately.
  - **Example:** Using adaptive Gaussian thresholding on a patch with varying illumination preserved essential features like inner and outer contours of myelin sheaths.



Global Thresholding (Inverted, Threshold: 140)   Adaptive Thresholding (Inverted)

Fig-4

As depicted in Fig-4, global thresholding often neglected smaller regions, which are potentially regenerating sites crucial for accurate analysis. Adaptive thresholding, by contrast, effectively captured these smaller, intricate details, making it the preferred method for this study.

Methodology

The core image analysis pipeline involved the following steps:

1. Edge Enhancement

Edge enhancement is a critical preprocessing step to highlight boundaries and improve object delineation in images. We explored multiple methods to identify the most effective technique for isolating myelin sheaths in neuro biopsy images. Below are the techniques tested, their processes, and outcomes:

a. Watershed Segmentation

- **Process**: This method treats the grayscale image as a topographic surface, where pixel intensities represent elevations. Watershed lines are identified to segment overlapping objects into distinct regions.
- **Application**: Suitable for separating closely packed objects or regions with overlapping boundaries.
- **Outcome**: While effective in segmenting overlapping structures, the method introduced additional complexity and failed to isolate myelin sheaths effectively due to the similarity in pixel intensities between overlapping and non-overlapping regions.

b. Canny Edge Detection

- **Process**:
  - Utilizes gradients to identify regions of rapid intensity change.
  - Applies two thresholds (low and high) to determine which edges to link, providing a clean binary edge map.
  - Includes steps like noise reduction (using Gaussian blur), gradient calculation, and edge linking.
- **Application**: Well-suited for images with clear boundaries and minimal noise.
- **Outcome**: The method struggled to identify inner contours clearly. Additionally, unnecessary noise was introduced in areas where myelin boundaries were faint or overlapped with background textures. This significantly impacted the effectiveness of downstream contour detection.

c. Sobel Edge Detection

- **Process**:
  - Computes the gradient magnitude of the image in both the x and y directions using the Sobel operator.

- **Application**: Effective for detecting prominent edges by emphasizing regions of high intensity change.
- **Outcome**: Sobel edge detection produced consistently good results. It was effective in highlighting prominent edges, especially in regions with high intensity changes. While it occasionally struggled with noise, the results were reliable for detecting outer and inner contours of myelin sheaths.

d. Bypassing Edge Enhancement

- **Process**: Skipped edge enhancement entirely, proceeding directly to thresholding and contour detection.
- **Application**: Avoided the potential over-processing of edges caused by traditional edge enhancement techniques.
- **Outcome**: Consistently provided the best results. Retaining the original features of the image ensured that segmentation remained robust and reliable, especially for detecting the intricate boundaries of myelin sheaths.

Comparison and Final Choice

Method	Strengths	Limitations
Watershed Segmentation	Handles overlapping regions effectively	Complex; struggled with subtle boundaries
Canny Edge Detection	Clear edge maps in low-noise regions	Did not detect Inner boundaries, Noise sensitivity; missed faint boundaries
Sobel Edge Detection	Enhanced sharp edges; simple computation	Introduced artifacts
Bypassing Edge Enhancement	Preserved original features; robust segmentation	None significant; best overall performance

Based on these experiments, **bypassing edge enhancement** was chosen as the most effective approach, enabling accurate contour detection without unnecessary artifacts or complexity. However, while Sobel was able to detect outer and inner contours accurately, it struggled to provide reliable thickness calculations due to artifacts or noise. This is where bypassing edge enhancement yielded better results.

2. Contour Detection

Contours, which represent boundaries of objects in the image, were extracted using OpenCV's `findContours` method. This step was critical for isolating and analyzing individual myelin sheaths and RBCs.

Process:

- The contours were detected from the thresholded images.
- Hierarchical relationships between contours (e.g., inner and outer boundaries of concentric shapes) were captured for accurate measurements of thickness.

Key Features:

1. **Inner and Outer Boundaries**:
    - Detected inner and outer contours of myelin sheaths to calculate thickness.
2. **Filtering Small Contours**:
    - Eliminated irrelevant contours based on area thresholds (e.g., contours smaller than 10 pixels) to reduce noise.
3. **Inner Contour Reference:**
    - To ensure precision in thickness calculation, the inner contour with the **maximum area** was identified. This inner contour served as the most stable and reliable boundary within the structure.

Metric Calculations:

1. **Diameter and Area**:
    - Computed the enclosed area and diameter within the contour.
2. **Thickness**:
    - To estimate the thickness of myelin sheaths, the difference between the radii of the outer and inner boundaries was calculated. This difference was then scaled by a factor to convert the pixel measurements to micrometers (µm\mu mµm):

      $\text{Thickness} = (\text{Outer Radius} - \text{Inner Radius}) \times \text{Scaling Factor (0.136 for µm)}$
    - Example:
        - Outer Radius = 15 , Inner Radius = 10.
        - Thickness = $(15-10) \times 0.136 = 0.68\mu m$.

3. RBC Detection

Red blood cells were identified and excluded to focus the analysis on myelin sheaths.

Process:

- Contours with no inner boundaries were classified as RBCs based on their shape and size.
- Patches were classified as RBC-dominant if the proportion of RBC contours exceeded a threshold (e.g., 70%).
- **Output**: Processed images were saved with annotated RBCs for validation and excluded from further analysis.

Results and Analysis

- Successfully detected and quantified myelin sheath structures and RBCs.
- Calculated:
  - Diameter of myelin sheaths, providing valuable insights into the size and uniformity of nerve fibers.
  - Thickness based on hierarchical contours, ensuring precise structural analysis.
- Classified RBCs accurately to ensure they were excluded from myelin analysis, thus avoiding interference in sheath evaluation.
- Enhanced segmentation results by employing adaptive thresholding, preserving critical details even in unevenly illuminated areas.
- **Example Images:**
  - Example of myelin sheath segmentation:

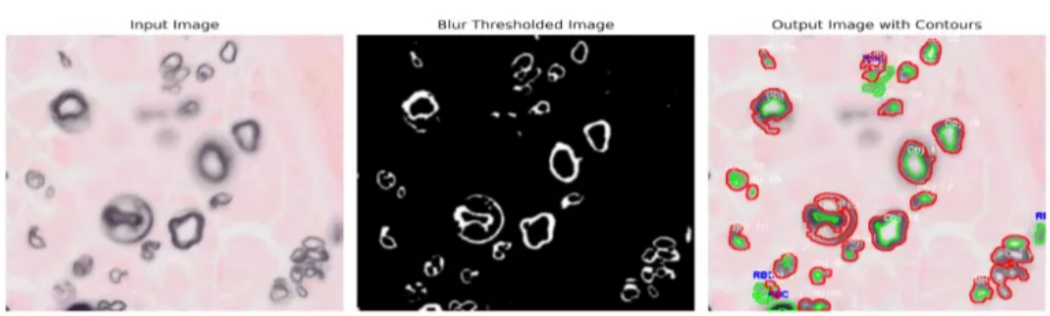


Fig-5

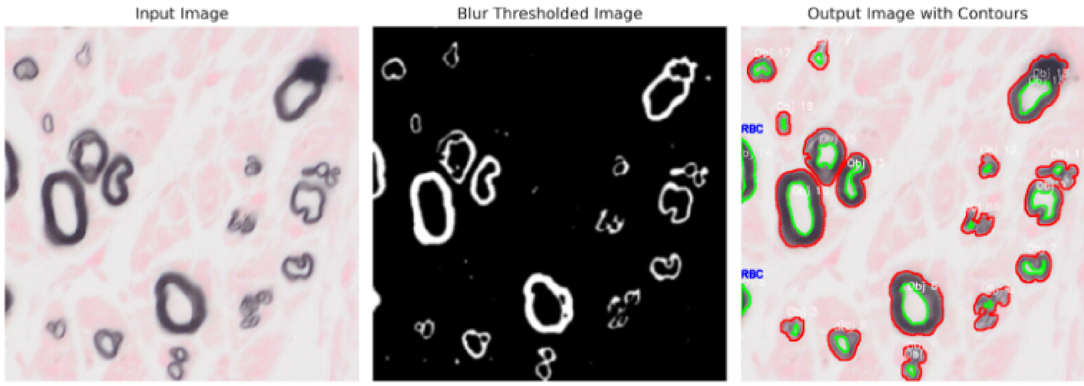


Fig-6

- Example of RBC classification:

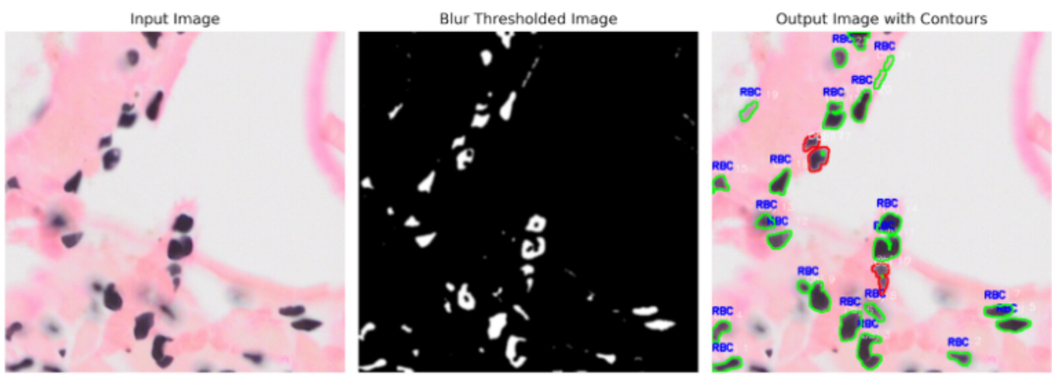


Fig-7

Conclusion

1. **Edge Enhancement Selection:**

    - Bypassing edge enhancement was chosen as the most effective approach, enabling accurate contour detection without unnecessary artifacts or complexity. While Sobel was able to detect outer and inner contours accurately, it struggled to provide reliable thickness calculations due to artifacts or noise. Additionally, Sobel was not able to detect RBCs effectively compared to bypassing edge enhancement. This limitation was effectively addressed by bypassing edge enhancement, which yielded better results.

2. **Preprocessing Improvements:**
    - Adaptive thresholding preserved critical details and improved segmentation quality.
3. **Contour Analysis:**
    - Focused on hierarchical relationships to isolate inner and outer boundaries of myelin sheaths accurately.
    - Quantified sheath thickness with precision using maximum area inner contours.
4. **Exclusion of RBCs:**
    - Reliable detection of RBCs ensured no interference in myelin analysis.

These advancements provide a foundation for further research into neurological disorders and the structural integrity of myelin sheaths.

References

1. OpenCV Morphological Operations Documentation
2. OpenCV FindContours Guide
3. OpenCV Contour Hierarchy Documentation
4. Dynamic Thresholding Discussion on StackOverflow

Git Link

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https://github.com/Vishnutha/Neural_Biopsy
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