Neurite Outgrowth Analysis Tool - User Manual

Version: v6.6
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Description: A GUI-based tool for neurite outgrowth analysis from microscopy images with adjustable ROI selection, overlay customization, and quantitative measurements in pixels and microns.

1. Introduction

This tool is designed to assist researchers in performing neurite outgrowth analysis from microscopy images. It enables precise Region of Interest (ROI) selection, adjustable detection parameters, and export of both results and annotated overlay images. It supports both pixel and micron measurements, scale bars, and customizable overlays.

2. System Requirements

Requirement	Windows	macOS
Python Version	Python 3.8 - 3.11	Python 3.8 - 3.11
Dependencies	opencv-python, numpy, pillow, tkinter, reportlab	opencv-python, numpy, pillow, tkinter, reportlab
Image Formats	PNG, JPG, TIFF	PNG, JPG, TIFF

3. Installation

1. Install Python from https://www.python.org/. 2. Open a terminal (Command Prompt on Windows, Terminal on macOS). 3. Install dependencies by running: pip install opencv-python numpy pillow reportlab 4. Save the neurite_analysis_gui_v6.6.py script to your desired directory.

4. Usage Instructions

1. **Load Reference Image**: Click 'Browse' next to 'Reference (0 h image)' to load your baseline image. 2. **Load Images to Analyse**: Select one or multiple images for analysis. 3. **Set Output Folder**: Choose where results and overlays will be saved. 4. **Adjust ROI**: Use 'Preview/Adjust ROI' to open an interactive window where you can drag polygon corners or move the polygon. 5. **Set Detection Parameters**: Adjust sliders for top-hat size, threshold offsets, minimum object size, and skeleton length. 6. **Overlay Settings**: Choose overlay color, thickness, and display mode (Color, Grayscale, B/W). 7. **Scale Bar Settings**: Enable and configure length, label, position, and color of the scale bar. 8. **Run Analysis**: Click 'Run Analysis' to process all images. 9. **Export Results**: Click 'Export CSV & Overlays' to save the quantitative table (pixels + microns) and annotated images.

5. Output Description

The tool generates: - CSV file containing image name, number of neurites, total length, maximum length, average length, and median length in both pixels and microns. - Overlay images highlighting detected neurites with chosen color and thickness. - Optional scale bar annotated on overlays.

6. Troubleshooting

- **Images not loading**: Check file format and ensure it's PNG, JPG, or TIFF. - **No neurites detected**: Adjust detection parameters, especially 'Min Area' and 'Min Length'. - **Overlay too faint**: Increase overlay thickness or choose a contrasting color.

7. License