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1. Introduction

MyeliMetric is a specialized application designed for neuroscience researchers working with myelin sheath data. It provides a comprehensive workflow for processing microscopy/EM measurements of axons and myelin sheaths, calculating fiber diameters and g-ratios, and performing statistical analyses to compare control (CTL) and experimental (EXP) samples.

Key Features:

- Automated calculation of fiber diameters and g-ratios
- Statistical analysis of myelin sheath characteristics
- Visualization through scatter plots and histograms
- Size-based categorization of nerve fibers
- Comparative analysis between control and experimental conditions

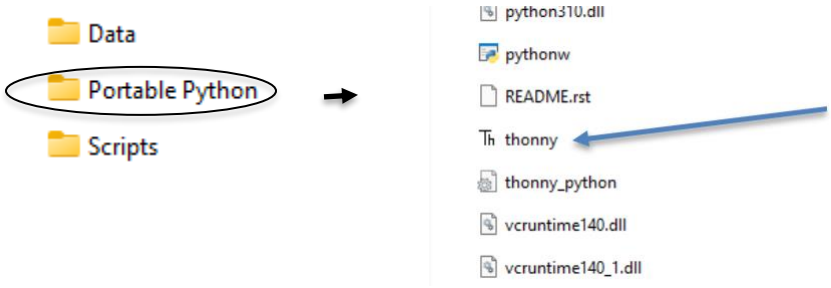
2. Installation and Run

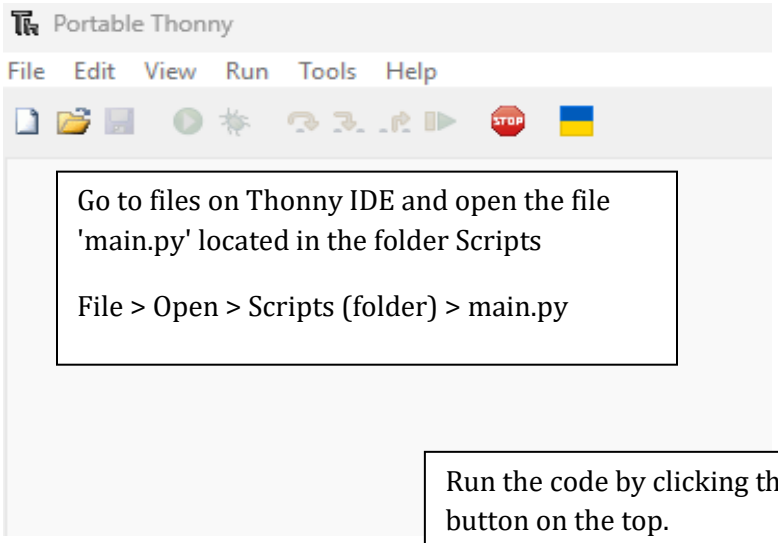
System Requirements:

- Windows, macOS, or Linux operating system
- 4 GB RAM minimum (8 GB recommended)
- 500 MB free disk space
- **Installation Option 1: Using the Portable Thonny Python Package (Recommended)**

Myelimetic User Manual

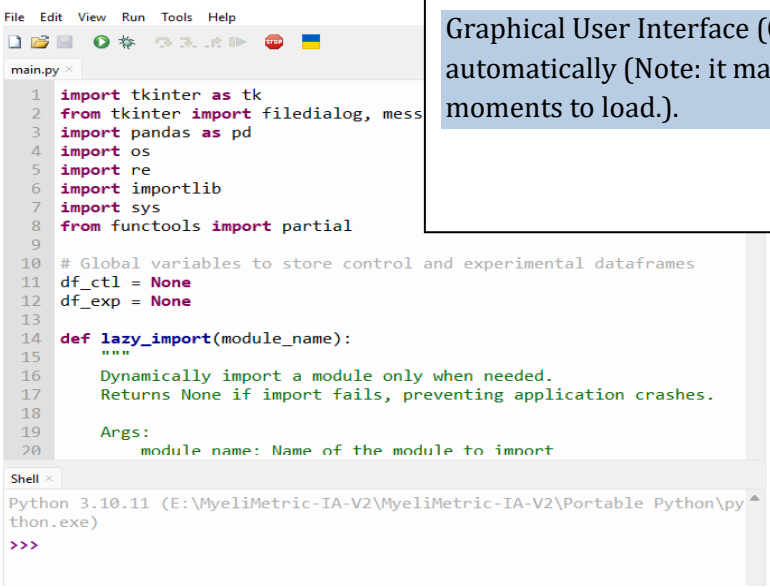
1. Download the ZIP file (‘No Python installation need (Windows only) ‘) from the GitHub repository to your computer and extract all files.
2. Use the bundled Thonny Python distribution with all dependencies preinstalled (portable).
3. Run Thonny, open script main.py from the Scripts folder, and execute to launch the Graphical User Interface (GUI).



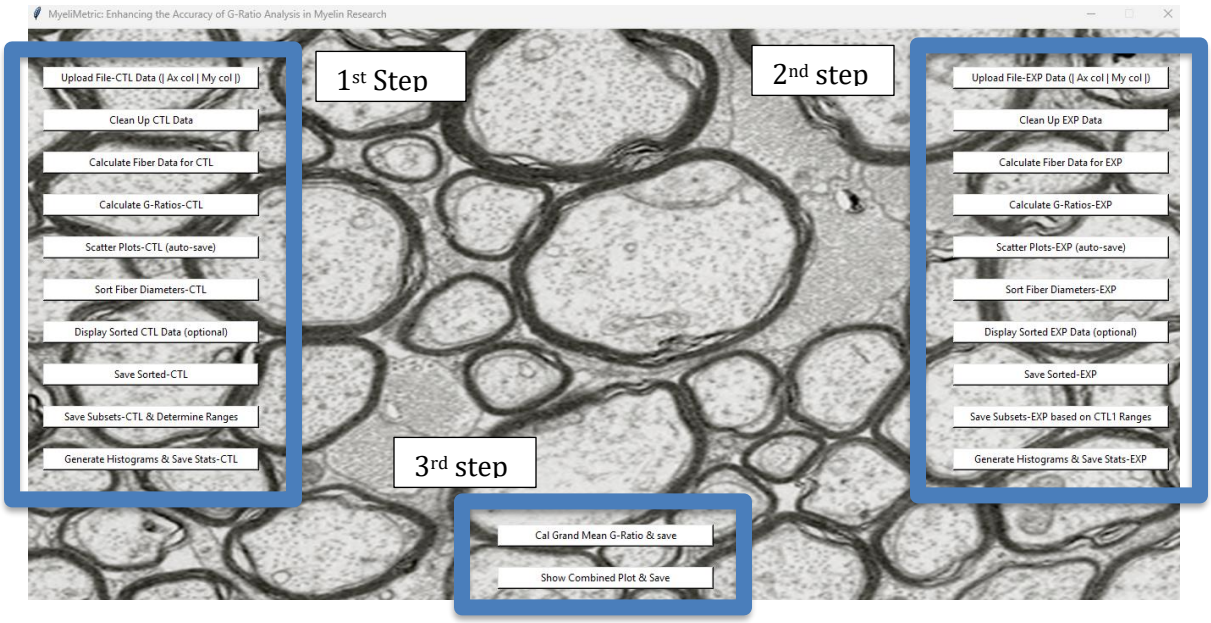


Run the code by clicking the green 'Run' button on the top.

Graphical User Interface (GUI) will open automatically (Note: it may take a few moments to load.).



GUI interface



Click each button in order, read the pop-up messages carefully, and monitor the Thonny IDE shell window.

After clicking a button, wait for the completion notification before proceeding.

Installation Option 2: Using Your Existing Python Installation/ IDE (Windows, macOS, or Linux)

Download the ZIP file (‘To run using a pre-installed Python IDE‘) from the GitHub repository to your computer and extract all files.

- Run the script: main.py located in the Scripts folder
- The GUI will appear

Note: When opening the application for the first time, begin by clicking the "Install All Dependencies" button located at the top-center of the GUI. The background image of the GUI may not be visible initially. For subsequent uses, you won't need to click this button again. This initial step ensures all the necessary dependencies are installed alongside the Python core modules.

Installation Option 3: Using Your Existing Python Installation/ IDE (Windows, macOS, or Linux)

Requirements:

- tkinter
- pandas
- numpy
- matplotlib
- openpyxl
- Pillow
- xlsxwriter

Install libraries via:

- pip install pandas numpy matplotlib openpyxl pillow xlsxwriter
- Download the ZIP file (‘No Python installation need (Windows only) ‘) from the GitHub repository to your computer and extract all files.
- Delete/ignore the portable Python folder, you don’t need it
- Then run the script: main.py located in the Scripts folder
- The GUI will appear

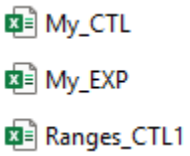
3. Getting Started

Prepare your Excel files with paired axon (_Ax) and myelin (_My) columns.
Example:

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	CTL1_Ax	CTL1_My		CTL2_Ax	CTL2_My		CTL3_Ax	CTL3_My		CTL4_Ax	CTL4_My		CTL5_Ax	CTL5_My
2	1.476033	0.072727		0.592905	0.095202		1.163845	0.277698		0.959969	0.971689		0.513667	0.665899
3	0.721041	0.095202		0.457185	0.054545		1.022205	0.23638		0.825186	0.602895		0.498158	0.367477
4	1.289881	0.290909		1.01647	0.184057		0.662733	0.135857		0.655709	0.424715		0.582472	0.653775
5	1.346118	0.39218		0.551585	0.090909		0.736363	0.113502		0.900014	0.904397		0.719884	0.282961
6	0.720657	0.201505		1.02649	0.090909		0.910605	0.127273		0.31677	0.254981		0.672948	0.138517
7	0.791892	0.240841		0.751726	0.109091		1.570695	0.286457		0.51462	0.301241		0.91223	0.981493
8	0.974359	0.343714		0.970183	0.090909		0.72301	0.142382		0.615948	0.699821		0.666802	0.915663

It is recommended to choose your highest-quality dataset for CTL1, as it defines the fiber-diameter ranges used for all subset creation.

Keep your data in the Data folder (it is recommended to name them like the following)



Note: It is mandatory to keep the ranges file in Data folder and name it exactly **Ranges_CTL1**, because this file will be updated with the range information.

Process CTL data first, then EXP data, and finally perform combined analysis.

4. Data Requirements

Input File Format: Microsoft Excel (xlsx or .xls) with matched axon/myelin columns.

- Column Naming Convention:
- Axon columns ending with "_Ax" (e.g., "Exp1_Ax")
- Myelin columns ending with "_My" (e.g., "Exp1_My")
- Base names must match each pair

5. Application Interface

The GUI has three sections:
- Left: Control (CTL) data processing buttons (Step 1)
- Right: Experimental (EXP) data processing buttons (Step 2)
- Bottom: Combined analysis buttons (Step 3)

6. Control Data Processing

Note: After clicking each button, wait for the completion pop-up to appear and then click OK before proceeding.

Upload File-CTL Data

Click “Upload File-CTL Data (| Ax col | My col |)”.
In the file dialog, navigate to and select your control Excel file (.xlsx/.xls).
On success, you’ll see “CTL file is successfully loaded!”—click OK to confirm.

Clean Up CTL Data

Click “Clean Up CTL Data” (The module removes any axon values < 0.15 µm or myelin values < 0.03 µm , thencompacts remaining measurements.)
When you see “CTL data cleanup is successful,” click OK.

Calculate Fiber Data for CTL

Click “Calculate Fiber Data for CTL”.
Computes each fiber diameter as (axon + myelin) and adds new columns FiberDiameter_<ExpName>.
Click OK when “Fiber data generation for CTL is successful.”

Calculate G-Ratios-CTL

Click “Calculate G-Ratios-CTL”.
Adds columns G-Ratio_<ExpName> = axon/(axon + myelin).
When “G-Ratio calculation for CTL is successful” appears, click OK.

Generate Scatter Plots-CTL

Click “Scatter Plots-CTL (auto-save)”.

Scatter plots of fiber vs. axon diameter are created, a linear fit is overlaid, and figures are saved to Scripts > ScatterPlot_CTL

After "scatter plots are saved successfully," click OK.

Sort Fiber Diameters-CTL

Click "Sort Fiber Diameters-CTL" (Data are sorted ascending by each FiberDiameter column.)

Click OK when the sorting completes.

Display Sorted CTL Data (optional)

Click "Display Sorted CTL Data (optional)".

A new window pops up showing the sorted table—close it when done to proceed.

Save Sorted-CTL

Click "Save Sorted-CTL".

In the save dialog, navigate to your Data folder, name the file (e.g., Sorted-CTL.xlsx), and click Save.

Confirm the "data saved successfully" message with OK.

Save Subsets-CTL & Determine Ranges

Click "Save Subsets-CTL & Determine Ranges".

Step 1: You'll be prompted to save the subsets workbook—choose your Data folder and name it Subsets-CTL.xlsx.

Step 2: You'll then be prompted to select (or create, if not present) an empty Ranges_CTL1.xlsx file—this will be populated with each subset's min/max fiber diameters. **Any preexisting data in that file will be overwritten.**

After both steps are complete, click OK on the final confirmation.

Generate Histograms & Save Stats-CTL

Click "Generate Histograms & Save Stats-CTL" (The module writes summary statistics to that file and saves frequency histograms).

When asked, open Subsets-CTL.xlsx.

Next, choose a save path in Data folder. Name the file (e.g., Stats-CTL.xlsx).

Histograms are automatically save in Scripts > Histograms folder

Click OK once the process finishes

7. Experimental Data Processing

All of the first eight buttons function the same way as those in the Control Data Processing section.

Save Subsets-EXP & Assign Ranges

Click "Save Subsets-EXP based on CTL1 Ranges."
When prompted load the **Ranges_CTL1.xlsx**. This file contains the six min/max fiber-diameter bins you generated from your control data.
After loading the ranges, you'll be prompted to save the experimental subsets. Navigate to your Data folder. Name it and Save (e.g., Subsets-EXP.xlsx), then click Save.
When the process finishes successfully, a pop-up will say "EXP subsets saved."
Click OK to proceed to the next step.

Generate Histograms & Save Stats-EXP

Similar to the previous processing section. EXP histograms will include the suffix "_EXP" in their filenames to distinguish them from the CTL histograms.

8. Combined Analysis

Note: As always, wait for each success message before moving on.

Calculate Grand Mean G-Ratio & Save

Click "Cal Grand Mean G-Ratio & save". Select Subsets-CTL.xlsx, then Subsets-EXP.xlsx, and finally choose file name (e.g., Combined G-ratios.xlsx) to save in Data folder.
The tool merges data, computes grand-mean g-ratios for CTL & EXP, and saves to the chosen file.

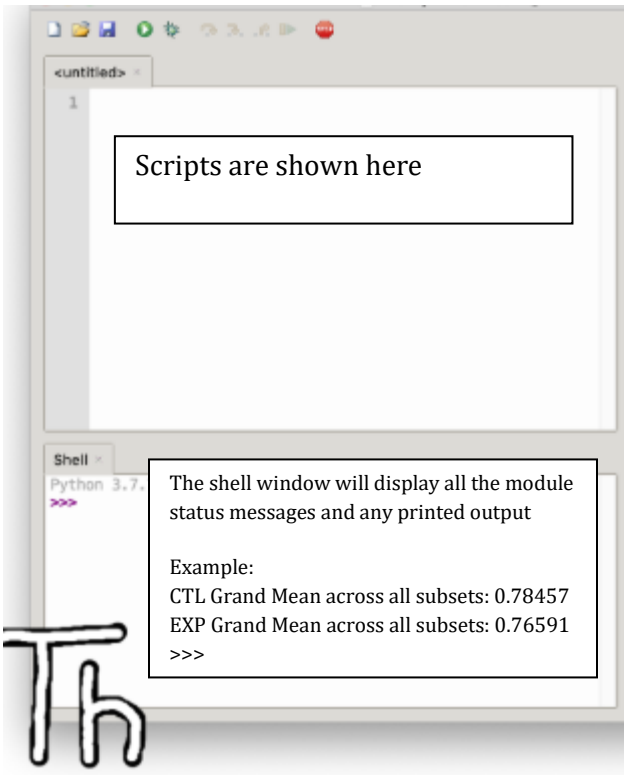
Show Combined Plot & Save

Click “Show Combined Plot & Save”.
Open Combined G-ratios.xlsx.
A comparative error-bar plot appears; Resize the plot window by dragging its edges vertically or horizontally to achieve the desired appearance.
close the window when you’re done.

The figure is saved in Scripts > Plots > CTL_EXP_plot.png.

Or you can save it to the location of your choice by clicking the 'Save' option on the display panel. It will save exactly what appears on the panel, so adjust it (since the sides of the panel are adjustable by dragging them with the cursor.) as needed to ensure all data points are clearly visible and there is no overlap.

The grand mean for CTL and EXP across all subsets will be shown in the Python shell window.



9. Output Files

All output .xlsx files are stored in the Data folder.

Sorted Data Files:

Sorted-CTL.xlsx
Sorted-EXP.xlsx

- Subset Files:

  Subsets-CTL.xlsx
  Subsets-EXP.xlsx

- Range File:

  Ranges_CTL1.xlsx

- Individual Statistical Analysis Files (CTL and EXP):

  Stats-CTL.xlsx

Stats-EXP.xlsx

- Combined Statistical Analysis Files (CTL and EXP):

  Combined G-ratios.xlsx

- Visual Output:
  All output figures are stored in the Scripts folder.

  ScatterPlot_CTL folder
  ScatterPlot_EXP folder
  Histograms folder
  Plots folder

10. Troubleshooting

- File Loading Errors: Ensure the Excel file is closed and properly formatted.

- Missing Experiment Columns: Verify column naming convention is correct.

- Module Not Found Errors: Confirm all scripts are in the same directory.

- Processing Order Errors: Follow steps in the correct sequence.

- Plot Display Issues: Check output folders for saved plots.

11. Glossary

Axon (Ax): Long projection of a neuron conducting electrical impulses.

Myelin (My): Fatty insulation layer around axons.

Fiber Diameter: Total diameter including axon and myelin.

G-Ratio: Axon diameter divided by fiber diameter.

CTL: Control sample data.

EXP: Experimental sample data.

Subset: Group of fibers by diameter range.