User Guide: Basement Membrane Annotation & GBM Profiling Macro (ImageJ)

📋 Overview

This macro allows users to annotate basement membrane boundaries in .tif images using the freehand tool. It then:

- Saves annotations as ROI zip files,
- Creates a binary mask,
- Calculates basement membrane thickness (via distance map),
- Skeletonizes the membrane,
- Computes width values,
- Saves the results as heatmaps and CSV profiles.

🛠️ Requirements

- ImageJ or Fiji
- Images in .tif format
- Calibration set in microns (optional, but recommended)

📂 Input Parameters (Prompted Automatically)

When the macro runs, it will prompt the user to specify:

1. **Input Directory** – Folder containing .tif images to process.
2. **Output Directory** – Folder where results will be saved.
3. **File Suffix** – Usually .tif (default).

🧭 Step-by-Step Instructions

1. **Launch ImageJ/Fiji** and open the macro (Plugins > Macros > Run...).
2. **Select the input/output directories** and confirm the file suffix.
3. **For each image**:
    - You'll be prompted to annotate the **basement membrane** using the **Freehand tool**.
    - After drawing, click **OK**.
    - You'll be asked if you want to annotate **more regions in the same image**.
        - Click **Yes** to draw more, or **No** to finish the image.

- Annotations will be saved as [filename]_BM.zip.

4. The macro will then:
    - Combine all ROIs
    - Generate a binary mask and compute the **distance map (heatmap)**.
    - Skeletonize the membrane.
    - Calculate **GBM width** as pixel/micron values.
    - Save:
        - Heatmap image: [filename]_HeatMap.tif
        - Final GBM width image: BM Width Image
        - Width profile as CSV: [filename]_GBMprofile.csv
5. You will be asked if you want to proceed to the **next image**.

💾 Output Files

For each input image, the following files are saved:

- *_BM.zip → Basement membrane ROI annotations
- *_HeatMap.tif → Distance map of annotated membrane
- *_GBMprofile.csv → Width values of basement membrane
- BM Width Image (in memory only unless saved manually)

📝 Notes

- Ensure that the **pixel size is set correctly** in the image metadata; otherwise, the macro defaults to pixels.
- For precise GBM thickness (in microns), both pixel width and height must match and unit must be "microns".
- The macro performs basic file management (closes images after processing).
- Skeletonized membranes are scaled to generate a pixelwise width profile.