Primary Objective

The tool processes microglia Regions of Interests (ROI) from microscopy image stacks and produces **quantitative morphology metrics** alongside **validated visual artifacts**, enabling reproducible, per-cell analysis across animals and imaging sessions.

Filename	ROI_Num	Number	Total Area	Mean Bra	Branch De	Cell Body Size		sk
1039\Ani	1	100	2189	21.89	15	2712		x
1039\Ani	2	48	879	18.3125	16	1282		x
1039\Ani	3	62	1783	28.75806	10	3978		x
1039\Ani	1	18	868	48.22222	7	1713		x
1039\Ani	2	21	450	21.42857	5	2008		x
1039\Ani	3	41	591	14.41463	10	1555		x
1039\Ani	1	126	2787	22.11905	15	2140		x
1039\Ani	2	42	851	20.2619	11	826		x
								not
1039\Ani	4	121	2723	22.50413	26	1643		x
1039\Ani	1	6	604	100.6667	3	1719		x
1039\Ani	2	114	2260	19.82456	17	3163		x

Tabular Output (Primary Quantitative Result)

File format

- **CSV or Excel (.xlsx)**

- One row **per ROI (single microglial cell)**

Required columns

Column Name	Description
Filename	Base image identifier (e.g., animal ID or acquisition name). Repeats for multiple ROIs from the same file.

ROI_Num   Sequential ROI index *(1…n)* within a given file. Resets when moving to a new image file.

Num_Branch_Points   Total number of skeleton branch points detected in the ROI.

Total_Area   Total segmented microglia area (pixels or µm²; units explicitly documented).

Mean_Branch_Length   Mean length of skeleton branches per cell.

Branch_Depth   Maximum or mean branching depth (clearly defined and consistent).

Cell_Body_Size   Soma size (area or equivalent diameter).
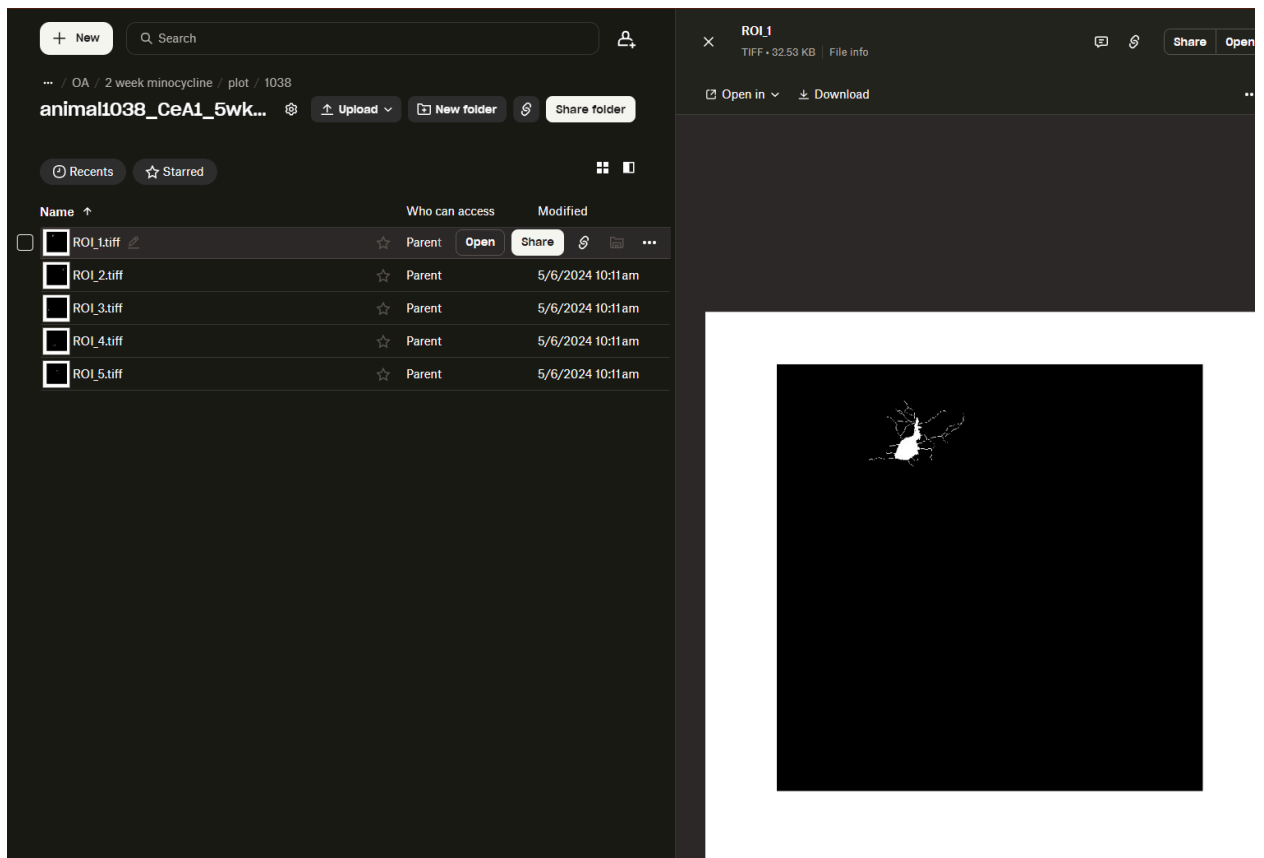
Skeletonized Image

- Skeleton representation derived from the binary mask

- Used for branch counting and morphology metrics

- Saved as: <Filename>_ROI<ROI_Num>_skele.tif

```
>Raw Data Directory >/
├───RawData/
│         ├── animal_123456
│         │         ├── cohortnum_subject123456_random01_zStack.czi
│         │         ├── cohortnum_subject123456_random02_zStack.czi
│         │         └── cohortnum_subject123456_random03_zStack.czi
│         │
│         ├── animal_123457
│         │         └── cohortnum_subject123457_random01_zStack.czi
│
```

```
>/Root_Output_Directory>/
│
├── ROI Save/
│         ├── morph_ROI_stats_subject123456.xlsx
│         └── morph_ROI_stats_subject123457.xlsx
│
│
│
│
├───GlialSkeletonPlots/
│         ├── animal_123456
│         │         ├── subject123456_random01
│         │         │         ├── ROI_1_skele.tiff
│         │         │         └── ROI_2_skele.tiff
│         │
│         │         ├── subject123456_random02
│         │         │         ├── ROI_1_skele.tiff
│         │         │         ├── ROI_2_skele.tiff
│         │         │         ├── ROI_3_skele.tiff
│         │         │         └── ROI_4_skele.tiff
│         │
│         │         └── subject123456_random03
│         │                   └── ROI_1_skele.tiff
│         │
│         ├── animal_123457
│         │         └── subject123457_random01
│         │                   ├── ROI_1_skele.tiff
│         │                   └── ROI_2_skele.tiff
│
```