**SkelAnal normalization and fragment removal protocol**

3/30/20 – updated 5/4/21

1. For each skeleton analysis raw data csv file (e.g. 6\_LeftDG\_1 or 40\_RightCA1\_2), order data in descending order by # branches (column A)
   1. Each row should represent one cell in an image
   2. “Fragments” that were identified separately by the skeleton analysis plugin that are not actual cells will be at the bottom and real cells with be at the top
2. Remove “fragments” from data file by deleting all data after the corresponding image’s cell count (found in “Hipp\_cell\_count.xlsx”)
   1. If an image has 24 microglia, all rows after row 24 will be deleted
   2. Each row represents 1 image
3. Calculate the sum of the remaining values in each of the following columns:
   1. # Branches (column A)
   2. # Junctions (column B)
   3. # End-points (column C)
   4. Average branch length (column F)
4. Normalize each measure by the corresponding cell count for the image by calculating the sum/cell (average) to give you values for the following:
   1. Branches/cell
   2. Junctions/cell
   3. End-points/cell
   4. Average branch length/cell