# Astrocytic intensity and area coverage analysis

Automatized pipeline

# Preprocessing

- Open ImageJ and open the macro *GLT-1_GFAP.ijm*

```
if (channels == 4) {
    run("Split Channels");
    selectWindow ("C1-" + title);
    run("Close");
    selectWindow ("C2-" + title);
    run("Close");
    selectWindow ("C3-" + title);
    rename("GLT");
    selectWindow ("C4-" + title);
    rename("GFAP");
```

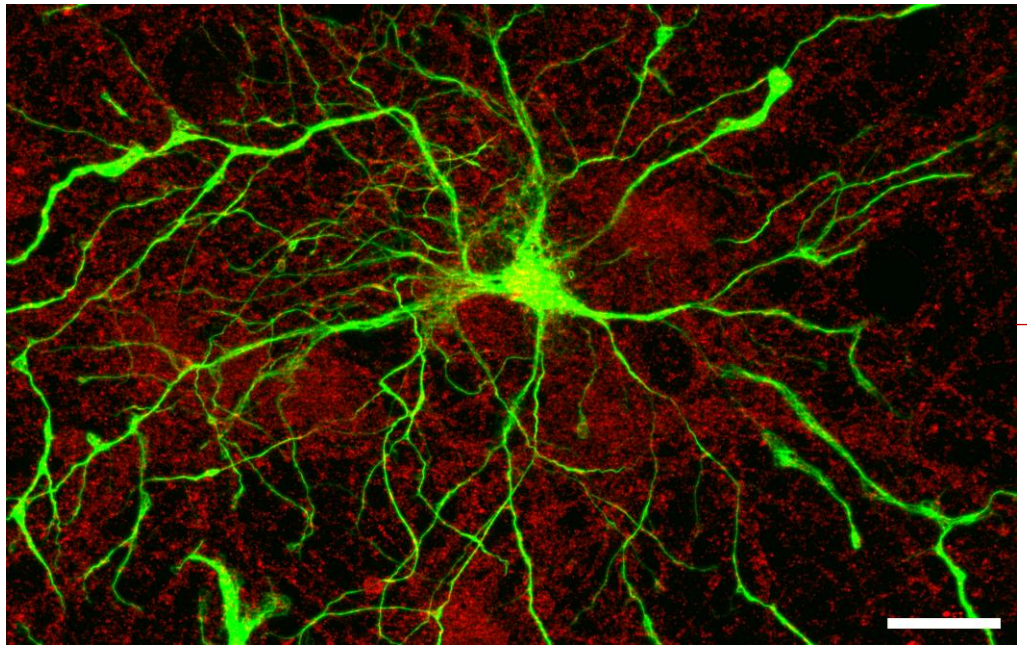
Please change channel name of GFAP and GLT depending on which channel hold this information.
If you have less than 4 channel, change it in the accroding line.

# Preprocessing

```
// Thresholding of Astrocytes
selectWindow("GFAP_threshold");
setAutoThreshold("Huang dark");
setOption("BlackBackground", false);
run("Convert to Mask");
selectWindow("GFAP_threshold");
run("Create Selection");
```

If your images are not good with the same thresholding algorithm, you can change the algorithm used here.

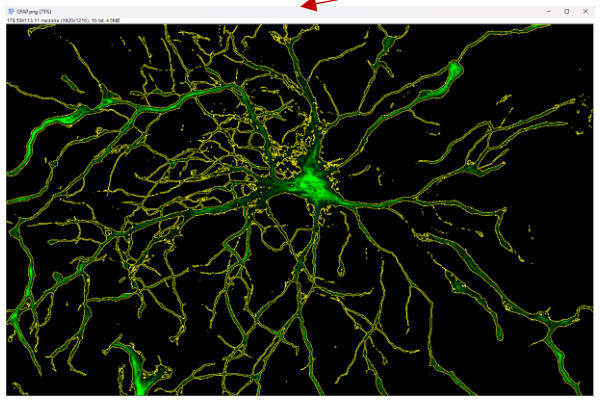
# Preprocessing

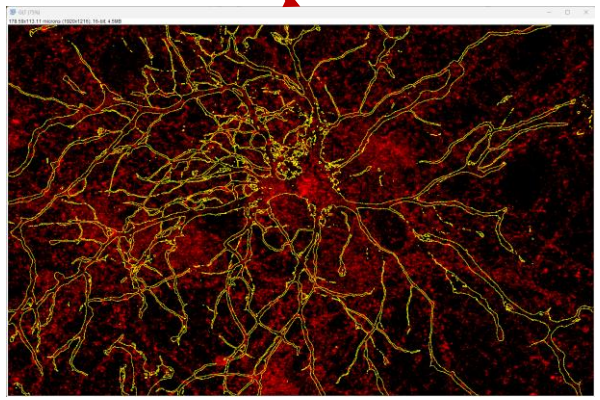


**thresholding**

Huang dark

**Selection overlay**





# Postprocessing of morphological data

- Use the *AstrocyteAnalysis.py* code to process you data

Important: you have to decode your data if they are coded

```
14    ##########################
15    #
16    # Please decode your conditions (or leave this empty if its already decoded)
17    #
18    ##########################
19    global replacements
20    replacements = {
21        'A1_' : 'Male_ctrl_24h_+MG_A_',
22        'A2' : 'Male_H7N7_24h_+MG_A_',
23        'A3' : 'Male_ctrl_24h_-MG_A_',
24        'A4' : 'Female_ctrl_24h_-MG_A_',
25        'A5' : 'Female_ctrl_24h_+MG_A_',
26        'A6' : 'Female_H7N7_24h_+MG_A_',
27        'A7' : 'Male_ctrl_6h_-MG_A_',
28        'A8' : 'Female_ctrl_6h_-MG_A_',
29        'A9' : 'Female_ctrl_6h_+MG_A_',
30        'A10' : 'Female_H7N7_6h_+MG_A_',
31        'A11' : 'Male_H7N7_24h_-MG_A_',
32        'A12' : 'Male_ctrl_6h_+MG_A_',
33        'A13' : 'Male_H7N7_6h_+MG_A_',
34        'A14' : 'Male_H7N7_6h_-MG_A_',
35        'A15' : 'Female_H7N7_6h_-MG_A_',
36        'A16' : 'Female_H7N7_24h_-MG_A_',
```

# Postprocessing of morphological data

If you would like to have an automatized normalization, you may have to adjust the round names (round_letter) and the time_points (if you have some)

```
132  ############################
133  #
134  # Normalization
135  #
136  ############################
137
138  df_data['Round'] = df_data['file'].str.extract(r'_([ABC])_')
139  df_data['Time'] = df_data['file'].str.extract(r'_([0-9]+h)_')
140
141
142  normalized_data = df_data.copy()
143
144  for round_letter in ['A', 'B', 'C']:
145      for time_point in ['6h', '24h']:
146          # Subset current group
147          current_group = df_data[(df_data['Round'] == round_letter) & (df_data['Time'] == time_point)]
```

# What you get after running the code

hyperexcitability.csv

hyperexcitability_normalized.csv

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | file | GFAP_area | GFAP_mean_ | GLT-1_mean_ | GLT-1_mean_int_whole | |
| 2 | MAX_Male_ct | 1312.83 | 199.186 | 77.386 | 64.779 | |
| 3 | MAX_Male_ct | 1979.437 | 110.76 | 61.517 | 52.438 | |
| 4 | MAX_Male_ct | 1292.429 | 362.056 | 74.476 | 65.262 | |
| 5 | MAX_Male_ct | 1996.127 | 384.861 | 69.125 | 54.002 | |
| 6 | MAX_Male_ct | 1523.756 | 387.29 | 77.027 | 67.689 | |
| 7 | MAX_Male_ct | 1826.739 | 309.369 | 81.972 | 66.208 | |
| 8 | MAX_Male_ct | 2076.322 | 421.51 | 71.03 | 58.234 | |
| 9 | MAX_Male_ct | 1519.664 | 114.974 | 79.808 | 63.738 | |
| 10 | MAX_Male_ct | 1951.751 | 260.464 | 70.393 | 56.434 | |
| 11 | MAX_Male_ct | 1849.32 | 238.218 | 83.268 | 65.808 | |

Despite the used intensity staining for GLT-1, each staining within astrocytes can be analyzed with this code!