Microglia and soma analysis

Expression profile via intensity staining and area analysis

Preprocessing Microglia

- Open ImageJ and open the macro *CCR5_MG_v001.ijm*

```
if (channels == 3) {
    run("Split Channels");
    selectWindow ("C1-" + title);
    rename("DAPI");
    selectWindow ("C2-" + title);
    rename("MG");
    selectWindow ("C3-" + title);
    rename("CCR5");


if (channels == 4) {
    run("Split Channels");
    selectWindow ("C1-" + title);
    rename("DAPI");
    selectWindow ("C2-" + title);
    rename("MG");
    selectWindow ("C3-" + title);
    rename("CCR5");
    selectWindow ("C4-" + title);
    run("Close");
```

Please change channel name of Microglia depending on which channel holds this information. And change the CCR5 channel depending on which chanel holds this information (can be any other staining, does not has to be CCR5)

Preprocessing Microglia

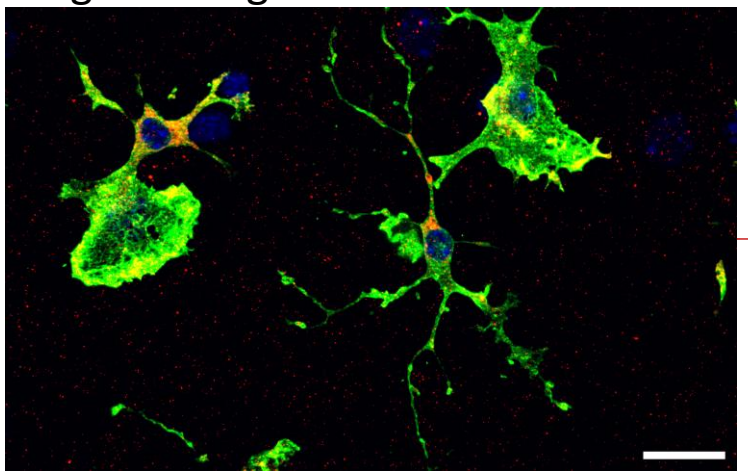
```
//IBA1 channel roi extraction
selectWindow("MG");
run("Enhance Contrast", "saturated=0.35");
run("Apply LUT");
setAutoThreshold("Li dark");
setOption("BlackBackground", false);
run("Convert to Mask");

waitForUser("Please correct the thresholding, then press ok.");
```

If your images are not good with the same thresholding algorithm, you can adjust the threshold algorithm.

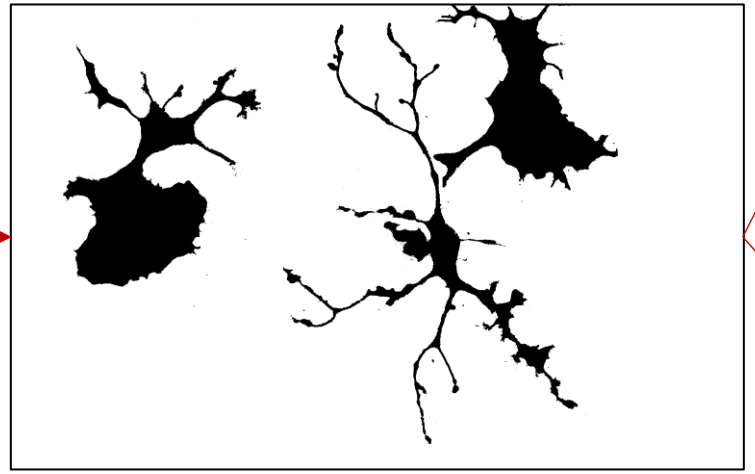
Your ask to correct the thresholdin in case it did not capture the entire truth of your image. Please be aware of NOT manipulating your data!

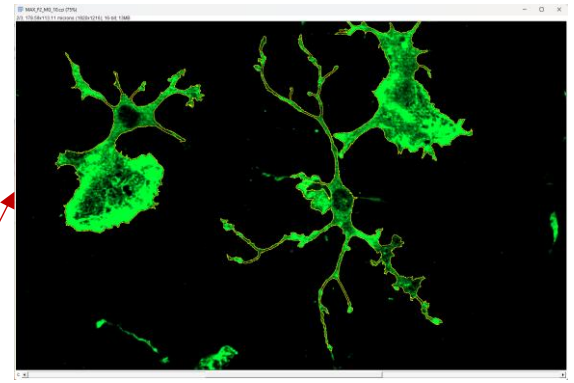
Example Microglia

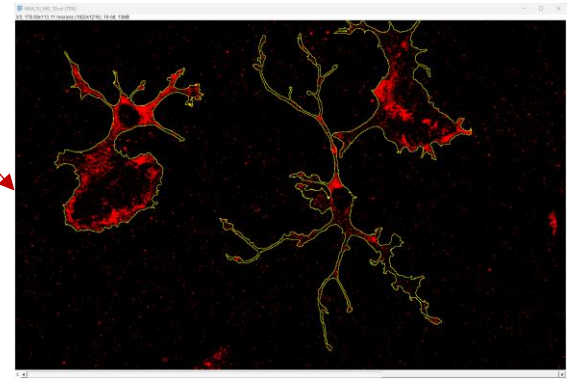
Original image



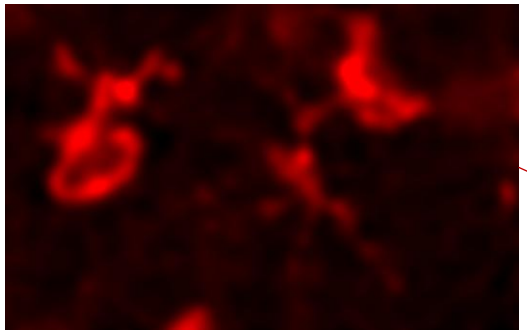
Li dark



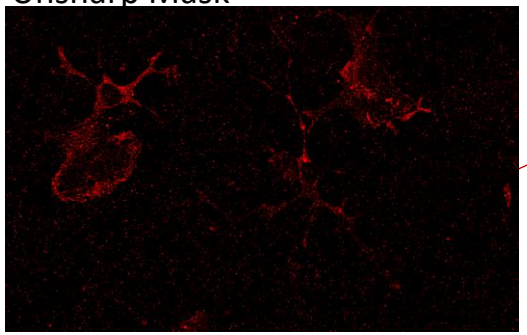


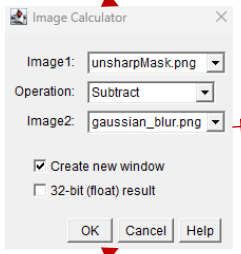


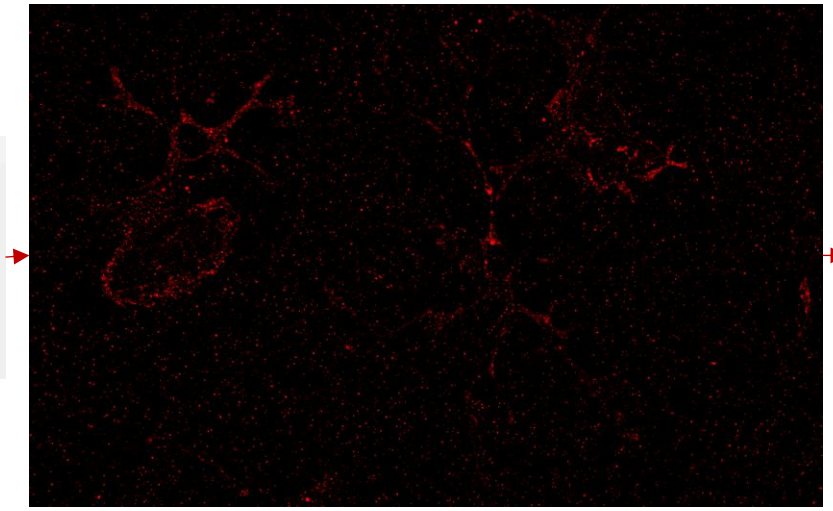
Gaussian blur



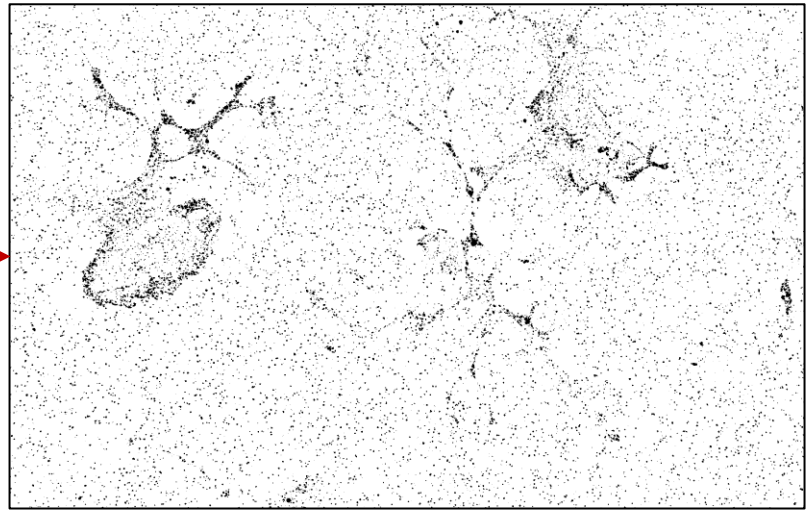
Unsharp Mask







Otsu dark



Postprocessing Microglia

Use the *CCR5_MG_v001.py* code to process you data

You need the following folder structure:

- folderWithAllConditions
  - ConditionA
    - RoundA
      - Images of RoundA
    - RoundB
      - Images of RoundB
    - …
  - ConditionB
    - RoundB
      - …

Change the code if needed to fit your data

```
##################################################
#
# Please enter your resolution!
#
##################################################

global resolution
resolution = 0.0930159

##################################################
#
# Please ensure the channel are correctly set
#
##################################################

CCR5_channel = 2
```

Enter the resolution of your images.

Define the channel of your channel to be analyzed.

What you get after running the code

	A	B	C	D	E	F	G	H	I	J	K	L
1	file	area_microglia	total_CCR5_area	CCR5_area_microglia	CCR5_puncta_microglia	CCR5_puncta_microglia_norm	total_CCR5_puncta	total_CCR5_puncta	total_mean_int	mean_int_microglia	total_IntDen	IntDen_microglia
2	MAX_A4_CCF	734.62042	5.60492913	0.07927404	573	0.77999465	13962	0.69119159	119.982865	282.149785	2423641.71	207272.994
3	MAX_A4_CCF	1053.1682	3.35229406	0.04180701	557	0.52888038	9660	0.47822022	98.5779387	194.406862	1991264.36	204743.125
4	MAX_A4_CCF	597.564759	3.39219525	0.0441745	349	0.58403712	9526	0.47158653	106.994213	244.818899	2161272.24	146295.146
5	MAX_A4_CCF	523.330963	4.08888192	0.0489692	291	0.55605347	11416	0.56515135	107.926119	211.848668	2180096.65	110866.967
6	MAX_A4_CCF	1129.98893	3.16526342	0.04538877	589	0.52124404	8989	0.44500223	95.6608034	190.728223	1932338.53	215520.781

Data obtained (for each condition)

- Area microglia
- Total CCR5 area (whole image frame)
- CCR5 area in microglia
- CCR5 punta in microglia
- CCR5 puncta in microglia per microglia area
- CCR5 puncta total (whole image frame)
- Total mean int (of CCR5 in whole image frame)
- Mean int (of CCR5) in microglia
- Total integrated density (of CCR5 in whole image frame)
- Integrated denstiy (of CCR5) in microglia

Preprocessing Neurons

- Open ImageJ and open the macro *CCR5_Neuron_v001.ijm*

```
if (channels == 3) {
     run("Split Channels");
     selectWindow ("C1-" + title);
     rename("DAPI");
     selectWindow ("C2-" + title);
     rename("CCR5");
     selectWindow ("C3-" + title);
     rename("NeuN");
if (channels == 4) {
     run("Split Channels");
     selectWindow ("C1-" + title);
     rename("DAPI");
     selectWindow ("C2-" + title);
     run("Close");
     selectWindow ("C3-" + title);
     rename("CCR5");
     selectWindow ("C4-" + title);
     rename("NeuN");
```

Please change channel name of Microglia depending on which channel holds this information. And change the CCR5 channel depending on which chanel holds this information (can be any other staining, does not has to be CCR5)

Preprocessing Neurons

```
//NeuN channel roi extraction
selectWindow("NeuN");
run("Enhance Contrast", "saturated=0.35");
run("Apply LUT");
setAutoThreshold("Otsu dark");
setOption("BlackBackground", false);
run("Convert to Mask");
run("Dilate");
run("Dilate");
run("Fill Holes");
run("Erode");
run("Erode");


waitForUser("Please correct the thresholding, then press ok.");
```

If your images are not good with the same thresholding algorithm, you can adjust the threshold algorithm.

To be able to obtain better thresholding and ROI results, the binarized image is dilated, holes are filled and afterwards eroded again. If it is not fitting for your data, please adjust here.

Your ask to correct the thresholdin in case it did not capture the entire truth of your image. Please be aware of NOT manipulating your data!

Postprocessing Neurons

Use the *CCR5_NeuN_v001.py* code to process you data

You need the following folder structure:

- folderWithAllConditions
  - ConditionA
    - RoundA
      - Images of RoundA
    - RoundB
      - Images of RoundB
    - …
  - ConditionB
    - RoundB
      - …

Change the code if needed to fit your data

```
##############################################
#
# Please enter your resolution!
#
##############################################

global resolution
resolution = 0.0930159

##############################################
#
# Please ensure the channel are correctly set
#
##############################################

CCR5_channel = 1
```

Enter the resolution of your images.

Define the channel of your channel to be analyzed.

What you get after running the code

	A	B	C	D	E	F	G	H	I	J	K	L
1	file	area_neuron	total_CCR5_area	CCR5_area_neuron	CCR5_puncta_neuron	CCR5_puncta_neuron_	total_CCR5_puncta	total_CCR5_puncta_norm	total_mean_int	mean_int_neuron	total_IntDen	IntDen_neuron
2	MAX_A4_CCR	653.966871	0.95559886	0.08786019	565	0.86395814	1640	0.08118853	66.3498025	182.257117	1340259.28	119190.117
3	MAX_A4_CCR	486.015069	0.83188537	0.09753623	464	0.95470291	1335	0.06608944	61.5284702	190.261803	1242868.86	92470.1033
4	MAX_A4_CCR	537.096227	3.60457255	0.07150681	468	0.87135224	8927	0.44193291	110.439634	249.800853	2230869.41	134167.096
5	MAX_A4_CCR	584.716602	7.49681982	0.10024859	755	1.29122381	17524	0.86752911	161.253014	288.21929	3257294.52	168526.604

Data obtained (for each condition)

- Area soma
- Total CCR5 area (whole image frame)
- CCR5 area in soma
- CCR5 punta in soma
- CCR5 puncta in soma per soma area
- CCR5 puncta total (whole image frame)
- Total mean int (of CCR5 in whole image frame)
- Mean int (of CCR5) in soma
- Total integrated density (of CCR5 in whole image frame)
- Integrated denstiy (of CCR5) in soma