



## MicroApp (beta version 1.1)

Tutorial: how to use the software

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**If you use this software for publication purposes, please cite:**

<https://doi.org/10.1016/j.nbd.2021.105449>

### What is MicroApp

MicroApp is a GUI software, designed to analyze in detail morphological characteristics from 3D reconstructed microglial cells, by using as its input excel files(.xls) produced by the Imaris software. For a detailed description of how MicroApp works and its results, please read on <https://doi.org/10.1016/j.nbd.2021.105449>.

*MicroApp is still in its beta version, so bugs or small dysfunctions might occur. Please report any problems you might face (see contact details in the link above).*

### Running the program

Before you run the program, make sure you have installed all necessary software/files as described in the *Prerequisites.pdf* file.

You can run the program through the JAVA executable jar file provided (*MicroApp for Windows.jar*) or download, extract and compile the source code (*MicroApp\_project.zip*) with a java compiler and run it from there.

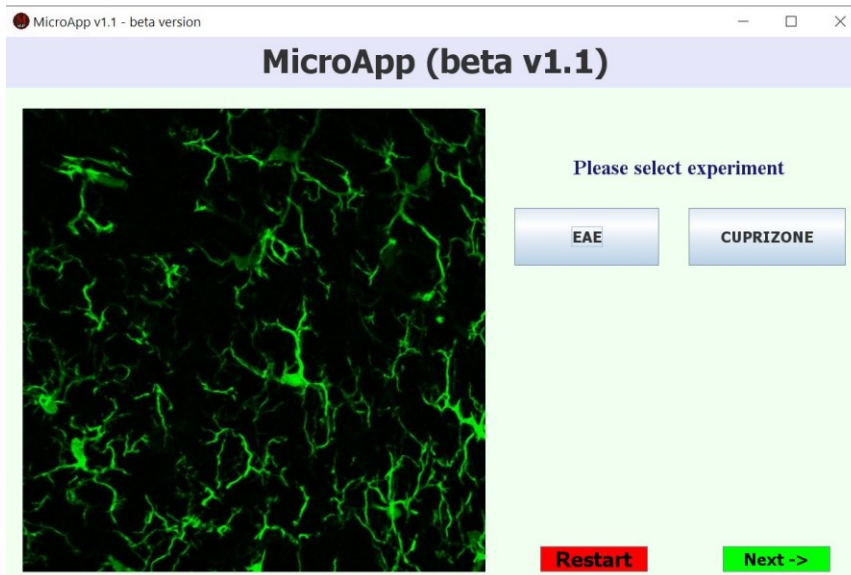
*\*This software has been developed and tested on a Windows OS. While it could work with macOS or other UNIX systems as well, minor adjustments might be needed in the source code for MicroApp to work properly. A working version for macOS will be uploaded soon!*

### Using MicroApp:

To provide a better understanding of how the program works, an example set of data is given in the *example.zip* file, so you should be able to reproduce the results as they are described in this tutorial. Please download the file and extract in a directory of your choice before reading further. The data includes 5 folders, each one with five excel files representing the data of five 3D reconstructed microglial cells corresponding to five different timepoints of a cuprizone experiment.

### Step 1:

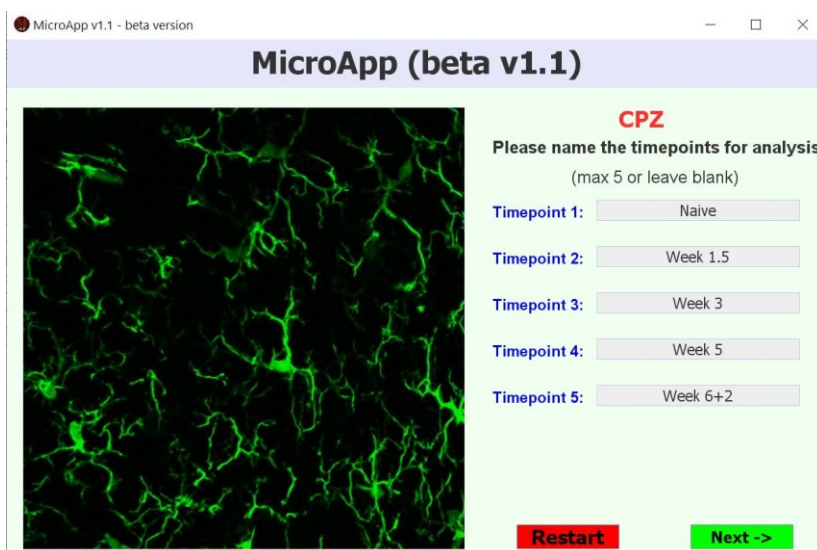
The initial screen will ask the user to choose between two experimental models, EAE and Cuprizone. Choose the experiment type of your choice and then click next.



The screenshot shows the initial screen of the MicroApp (beta v1.1). On the left is a large image of green fluorescent neurons on a black background. The title bar reads 'MicroApp v1.1 - beta version'. The main header is 'MicroApp (beta v1.1)'. Below the header, the text 'Please select experiment' is centered. There are two buttons: 'EAE' and 'CUPRIZONE'. At the bottom, there are two buttons: 'Restart' (red) and 'Next ->' (green).

### Step 2:

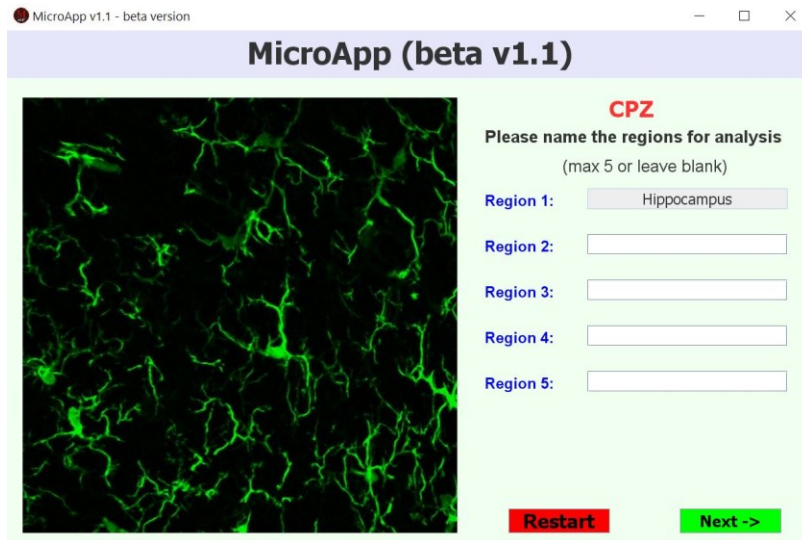
The user is asked to provide the labels for the timepoints of their experiment. Write the label next to each timepoint. If your timepoints are less than the textfields ( 5 for Cuprizone and 6 for EAE experiments), just leave blank. When you are ready , click next.



The screenshot shows the second screen of the MicroApp (beta v1.1). The title bar and header are the same as in Step 1. The text 'CPZ' is displayed in red. Below it, the text 'Please name the timepoints for analysis' is centered, followed by '(max 5 or leave blank)'. There are five text input fields labeled 'Timepoint 1:' through 'Timepoint 5:'. The values entered are: 'Naive', 'Week 1.5', 'Week 3', 'Week 5', and 'Week 6+2'. At the bottom, there are two buttons: 'Restart' (red) and 'Next ->' (green).

### Step 3:

The user is asked to provide the labels for the CNS regions that they want to analyze. Fill in the labels next to their text fields and click next. In this example, the cells are from the Hippocampus CA1 region.



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### MicroApp (beta v1.1)

**CPZ**

Please name the regions for analysis  
(max 5 or leave blank)

Region 1:

Region 2:

Region 3:

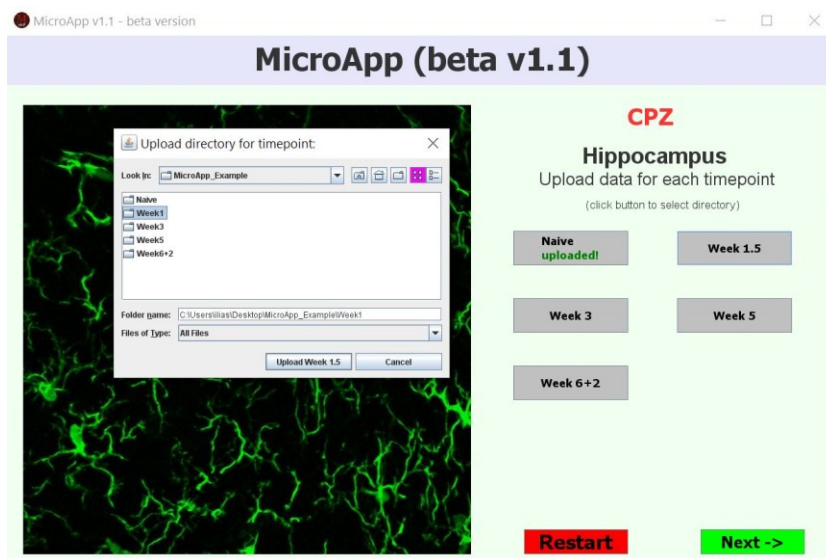
Region 4:

Region 5:

**Restart** **Next ->**

#### Step 4:

The user is asked to provide the data for each timepoint. Click on the button of each timepoint , a dialog window will appear so you can choose the correct directory. Choose the correct data for each timepoint, from the MicroApp\_Example folder. After you upload a timepoint, the green labeled text “OK!” will appear, indicating successful upload. **Important : make sure the timepoints are uploaded in sequence (naïve -> week 1.5 -> week 3 -> week 5 -> week 6+2 ).**



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### MicroApp (beta v1.1)

**CPZ**

**Hippocampus**

Upload data for each timepoint  
(click button to select directory)

**Naive** **Week 1.5**

**Week 3** **Week 5**

**Week 6+2**

**Restart** **Next ->**

**Upload directory for timepoint:**

Look in:

- Naive
- Week1**
- Week3
- Week5
- Week6+2

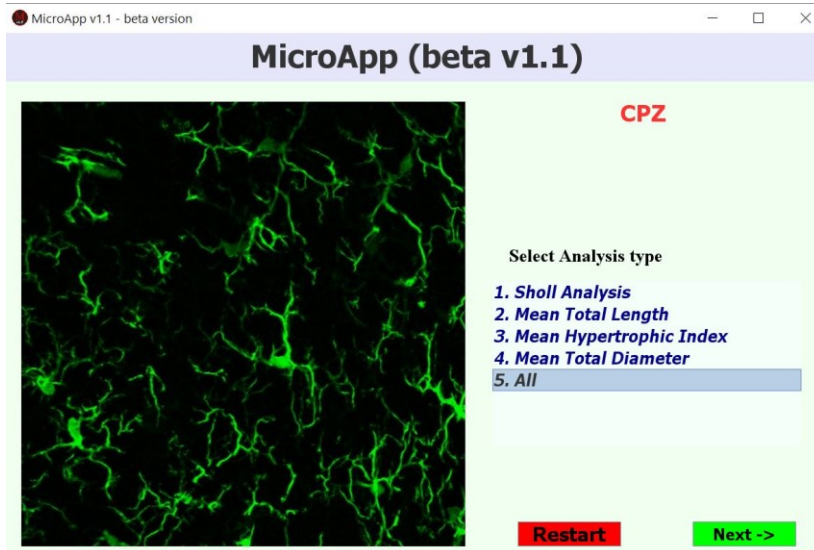
Folder name:

Files of type:

**Upload Week 1.5** **Cancel**

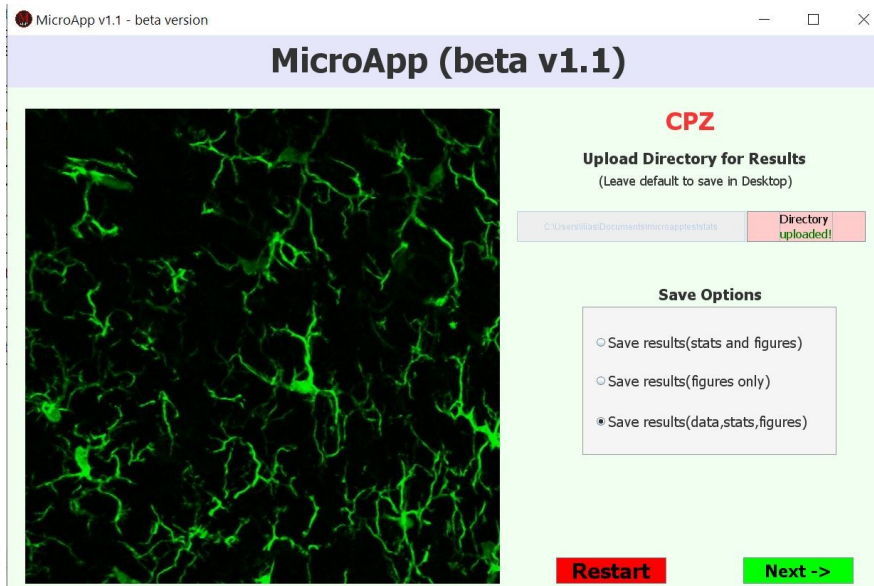
### Step 5:

The user is asked to choose the type of analysis they want to perform. You can choose among Sholl Analysis, Mean Process Length, Mean Hypertrophic Index, Mean Total Diameter or choose All to perform all 4 analyses ( **recommended!**). After choosing the analysis from the list, click next.



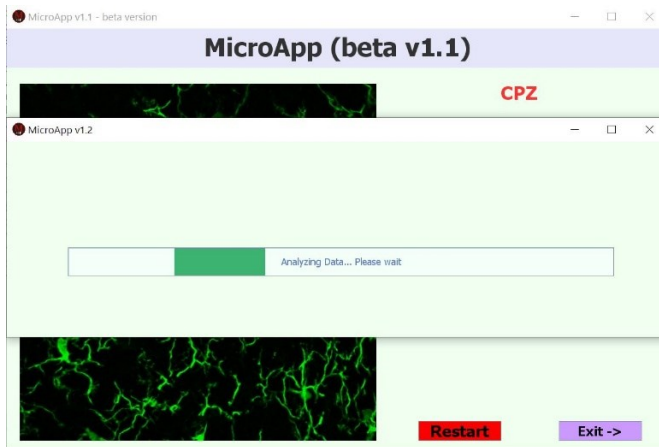
### Step 6:

The user is prompted to choose the directory where the results will be saved. If left blank, the results will be saved in the user's Desktop (not recommended). The user is also asked to choose one of the 3 output types. *If you don't own Matlab's Statistics and Machine Learning Toolbox (see Prerequisites.txt), then please choose the output "Save results (without stats)".* Click next.



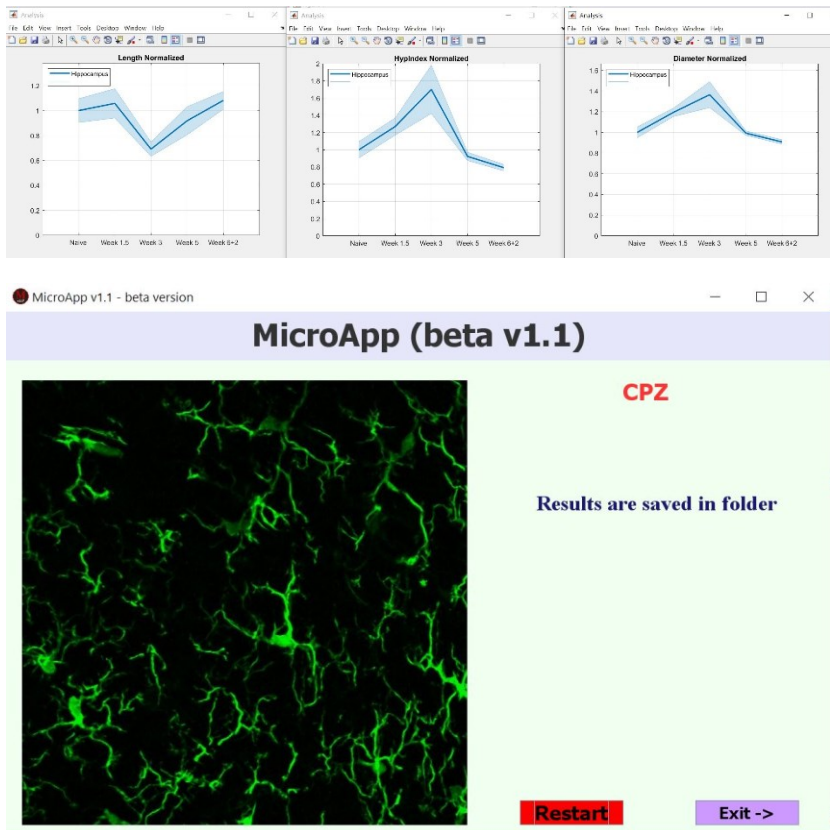
### Step 7:

Please wait while MicroApp analyzes the data and produces the final results. Depending on the size of your data or the specifications of your computer, this might take a few minutes.



### Step 8:

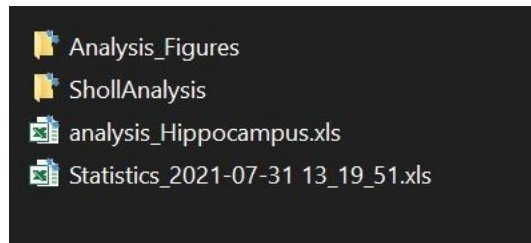
MicroApp will show the figures of the analyses and a screen informing you that the results have been saved. *Important: In this beta version, the restart button might not work properly. Please rerun MicroApp if you want to use it again. This bug will be fixed in later versions*



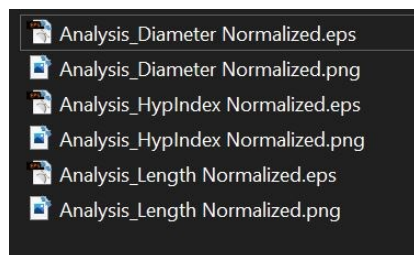


## Reading the Results

MicroApp can now be closed. Please browse to the result directory that you specified earlier (or desktop by default). MicroApp creates a results directory named MicroApp\_Results in the specified folder. If a previous analysis has been performed, in order to avoid overwriting your previous data MicroApp will create a new directory for the current analysis, with an incremental number in the title (e.g. MicroApp(1), MicroApp(2), etc... ). The results folder will contain the following files in the directory.



-The **Analysis\_Figures** folder contains the figures for the morphological analyses (Length, Hypertrophic Index and Diameter). The image files have been saved in two formats. **PNG** and **EPS**.



-The Sholl Analysis folder contains the figures for the Sholl Analysis, again in **PNG** and **EPS** formats.

-The **analysis\_Hippocampus** file contains the raw data for each analysis, cell by cell. It also provides the average over all cells for each timepoint, as well as the standard deviation and the standard error for these analyses.

HIPPOCAMPUS									
NAIVE		WEEK 1.5		WEEK 3		WEEK 5		WEEK 6+2	
Cell Name	Total Process Length	Cell Name	Total Process Length	Cell Name	Total Process Length	Cell Name	Total Process Length	Cell Name	Total Process Length
cell1_Naive.xls	751.31	cell1_W1.xls	598.28	cell1_W3.xls	353.56	cell1_W5.xls	550.64	cell1_W6+2.xls	640.04
cell2_Naive.xls	497.76	cell1_W2.xls	955.96	cell2_W3.xls	490.36	cell2_W5.xls	481.29	cell2_W6+2.xls	568.10
cell3_Naive.xls	569.55	cell1_W3.xls	661.75	cell3_W3.xls	386.67	cell3_W5.xls	577.21	cell3_W6+2.xls	749.05
cell4_Naive.xls	815.40	cell1_W4.xls	507.69	cell4_W3.xls	420.90	cell4_W5.xls	464.13	cell4_W6+2.xls	830.84
cell5_Naive.xls	562.28	cell1_W5.xls	659.05	cell5_W3.xls	556.57	cell5_W5.xls	863.58	cell5_W6+2.xls	671.60

	NAIVE	WEEK 1.5	WEEK 3	WEEK 5	WEEK 6+2
Average	639.26	676.55	441.61	587.37	691.93
St.Dev.	136.37	168.25	81.84	161.39	101.24
SEM	60.99	75.25	36.60	72.18	45.28

-Finally, the Statistics folder contains the statistical reports for the analyses. MicroApp performs normality tests and then provides the p-value based on the appropriate statistical test (t-test or non-parametric Mann-Whitney test). The type of test performed is mentioned in the third row of the results. The statistically significant p-values ( $p < 0.05$ ) are given in red background.

		Length			
		Week 1-5	Week 3	Week 5	Week 6+2
Naive		0.710	0.023967	0.598	0.508
test		t-test	t-test	t-test	t-test
		Hypertrophic Index			
		Week 1-5	Week 3	Week 5	Week 6+2
Naive		0.087	0.04571	0.492	0.077
test		t-test	t-test	t-test	t-test
		Diameter			
		Week 1-5	Week 3	Week 5	Week 6+2
Naive		0.01979	0.027877	0.901	0.142
test		t-test	t-test	t-test	t-test

*Following this tutorial, you should be able to reproduce the same results using the data of the example file.*

## **Reference**

Roufagalas I, Avloniti M, Fortosi A, Xingi E, Thomaidou D, Probert L, Kyrargyri V. Novel cell-based analysis reveals region-dependent changes in microglial dynamics in grey matter in a cuprizone model of demyelination. *Neurobiol Dis.* 2021 Jul 16;157:105449. <https://doi.org/10.1016/j.nbd.2021.105449> PMID: 34274460.