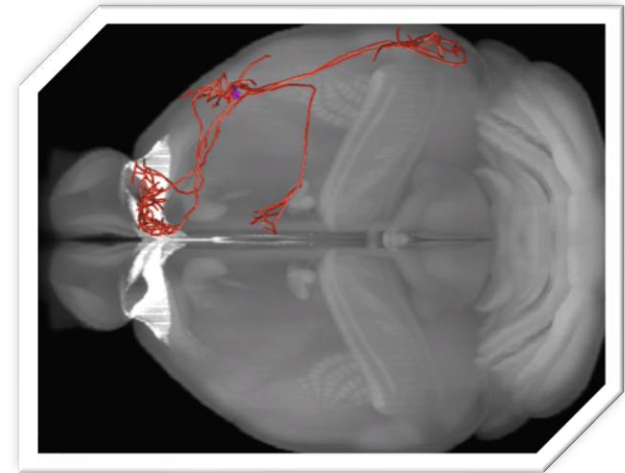


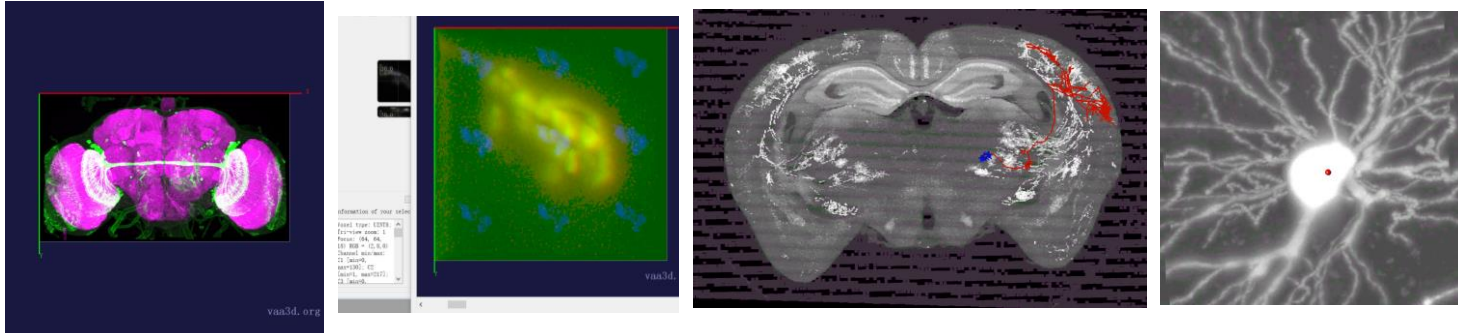
Reconstruction and quality control of neuron morphology in Vaa3D

Lulu Yin
20211207

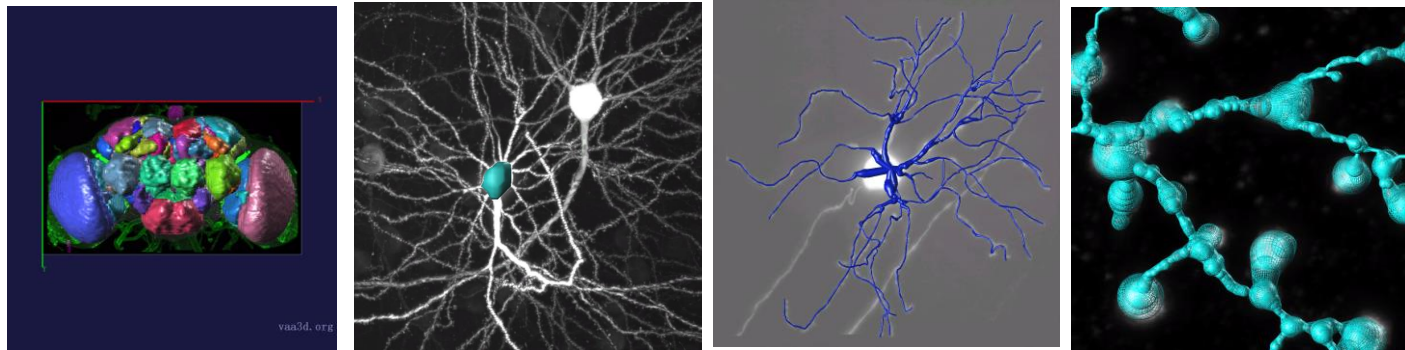


Vaa3D : The “3D Visualization-Assisted Analysis” software

- For the visualization and analysis of large-scale multidimensional images.



- Visualization of heterogeneous images and respective surface objects .

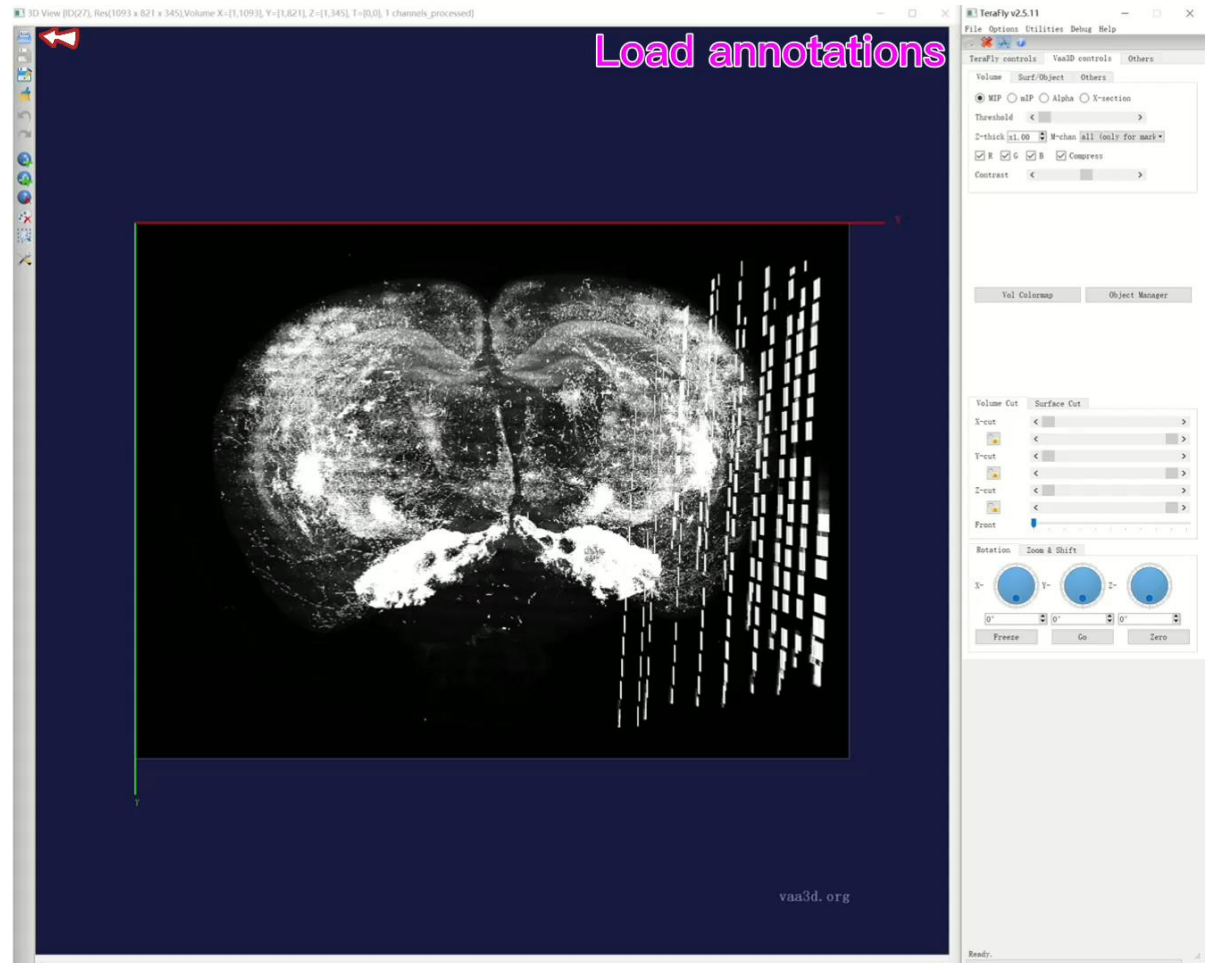


- Extension of Vaa3D functions using its plug-in interface.
- 3D image object generation and quantitative measurement.
- Neuron morphology reconstruction, quantification and comparison.

Vaa3D-TeraFly: Neuron morphology reconstruction

Video show: TeraFly

- Load annotations
- Annotate data
- Add marker
- Change resolution:
Double click or Zoom-in/out with mouse-scroll
- Volume cut
- Save annotations

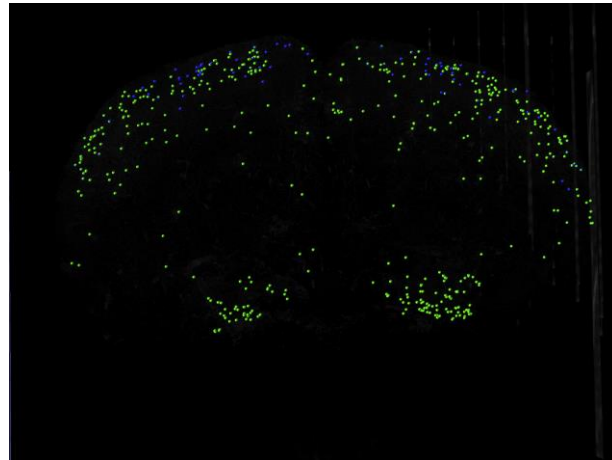
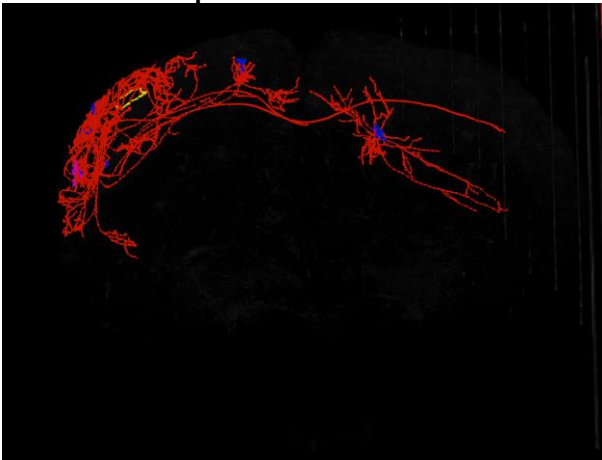


Expansion 1、Linker-file Plug-in

- Plug-in path

linker_file	▶ Linker_File_Generator	▶ for all SWC/ESWC (e.g. neuron) files under a directory
marker_utilities	▶ linker_teraflly	▶ for all APO (point cloud) files under a directory
misc	▶ Save_3Dviewer_Content_to_Linked_File	▶ for all TIF images under a directory
movies_and_snapshots	▶	▶ for all LSM images under a directory
neuron_toolbox	▶	▶ for all V3D Raw images under a directory
neuron_tracing	▶	▶ for all images (*.tif, *.tiff, *.raw) under a directory
neuron_utilities	▶	▶ for all V3D-recognizable files under a directory

- Linker_file_Generator: Link multiple SWC/ESWC, APO, TIF, LSM, etc. to the same directory, which can be opened in one brain at the same time.



- Linker_teraflly: Generate the corresponding ano and apo files from a single swc, so that the swc file can be opened in teraflly.

pre_18454_00305_YLL_LJ_YLL_LJ_stamp_2021_01_27_15_07.ano.swc
pre_18454_00305_YLL_LJ_YLL_LJ_stamp_2021_01_27_15_07.ano.swc.ano
pre_18454_00305_YLL_LJ_YLL_LJ_stamp_2021_01_27_15_07.ano.swc.apo

2021/1/27 22:51
2021/8/9 10:19
2021/8/9 10:19

Expansion 2、 Multiple swc files generate corresponding ano files in batches, and extract swc file information

- Use this script and enter the command line to generate ano files in batches

```
WJX@DESKTOP-IDNU9AG MINGW64 /d/SEUAllenJointDataCenter/MorphoHub/Database/Functions/Init (master)
$ sh ./GenerateAnoInfolder.sh /z/L2/YLL/swc ← swc file
/z/L2/YLL/swc/17786_0001/_YLL_stamp_2020_11_10_10_35.ano.eswc
/z/L2/YLL/swc/17786_00023_YLL_stamp_2020_11_10_10_34.ano.eswc
/z/L2/YLL/swc/17786_00024_YLL_stamp_2020_11_10_10_33.ano.eswc
```

Picture1

- Use the following script to extract swc information: brain and neuron numbers, neuron coordinates, neuron tracking author, neuron completion status and update time, etc.

```
WJX@DESKTOP-IDNU9AG MINGW64 /z/L2/YLL_WYP_trackinglist ← swc file
$ sh ./get_statistics_table.sh /z/L2/YLL_WYP_trackinglist/Get_data_Excel /z/L2/YLL_WYP_trackinglist/pre_reconstruction_somalist ← somalist path
pre_18864_00005
18864, 5332_X2880_Y20576,18864_00005,SEU,stamp,Finished,2021,2021_07_30_15_45
pre_18864_00007
18864, 5366_X3112_Y12350,18864_00007,SEU,wyp,Finished,stamp,2021_07_21_16_15
```

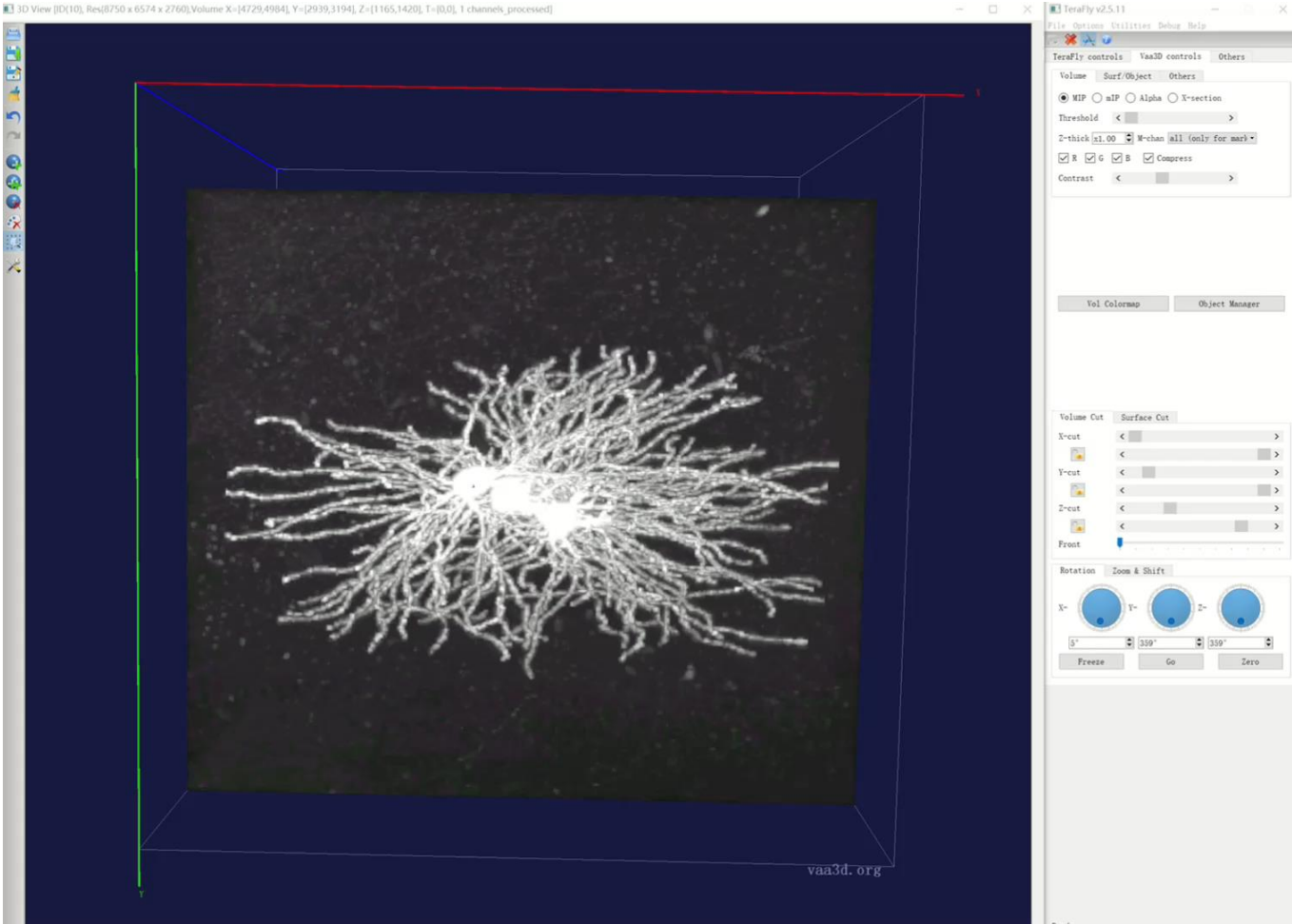
Picture2

Result:

Hust_Brain	Cellname	SEU_Neurc	Reconstruc	Reconstruc	Reconstruc	Reconstruc	Reconstruction_UpdateDate
18864	4610_X6120_Y30222	18864_001	SEU	YLL	Finished	stamp	2021_08_02_10_44
18864	5776_X7238_Y31018	18864_001	SEU	YLL	Finished	stamp	2021_08_02_10_45
18864	5208_X9666_Y1706	18864_002	SEU	YLL	Finished	stamp	2021_08_02_10_45

These two methods come from Shengdian

Expansion 3、 Method to improve reconstruction speed: manual and controllable automatic reconstruction

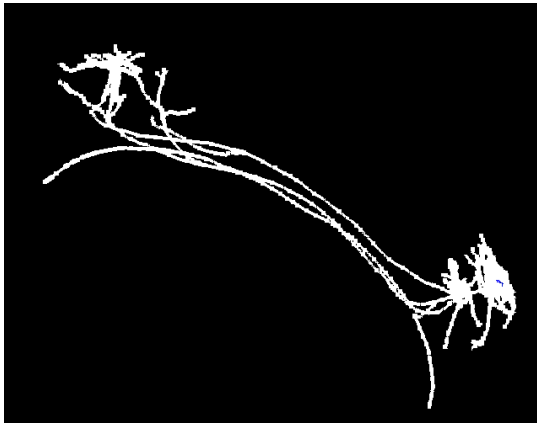


The retrace plug-in comes from Xuan Zhao

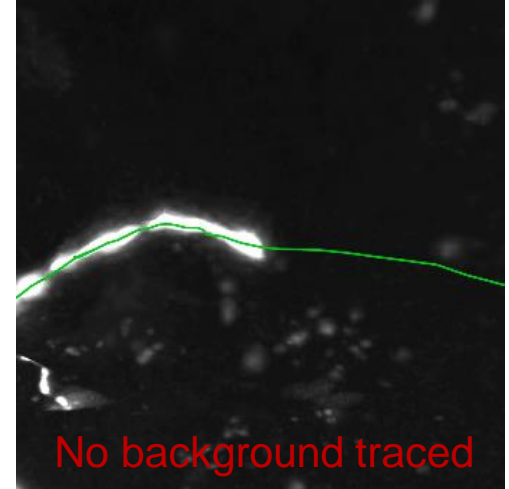
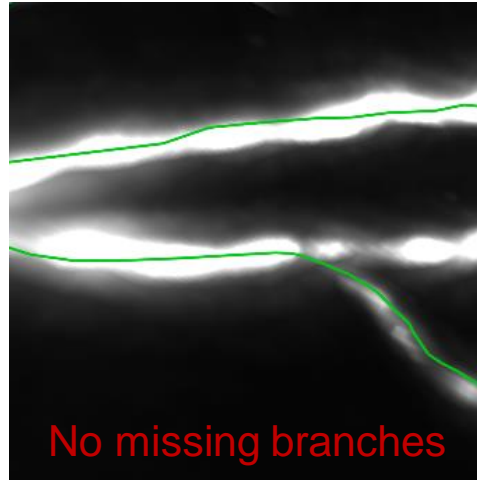
Vaa3D-TeraFly and TeraVR: Quality control of neuron reconstruction

Manual inspection:

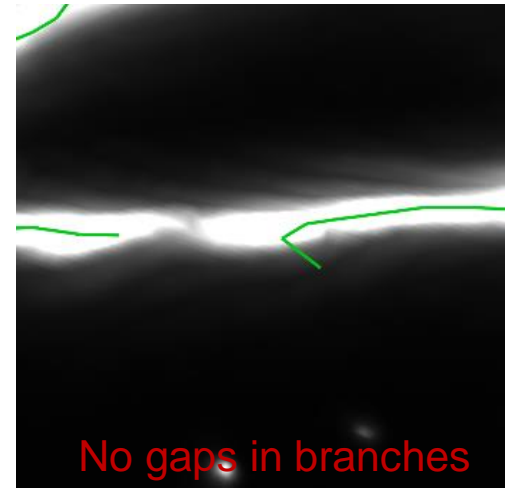
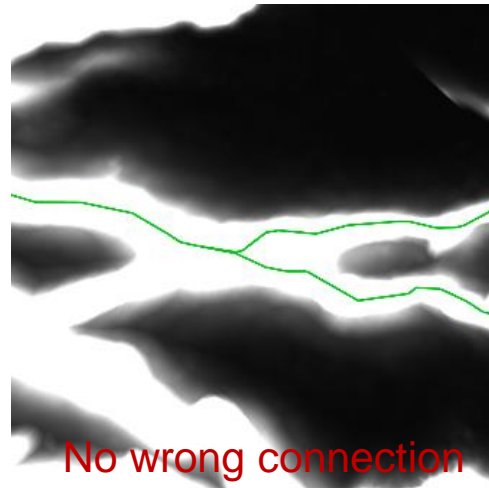
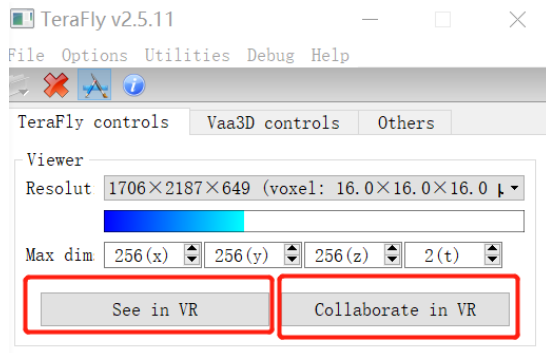
- Use Alt+N: A signal tree



- Need to meet the following requirements:

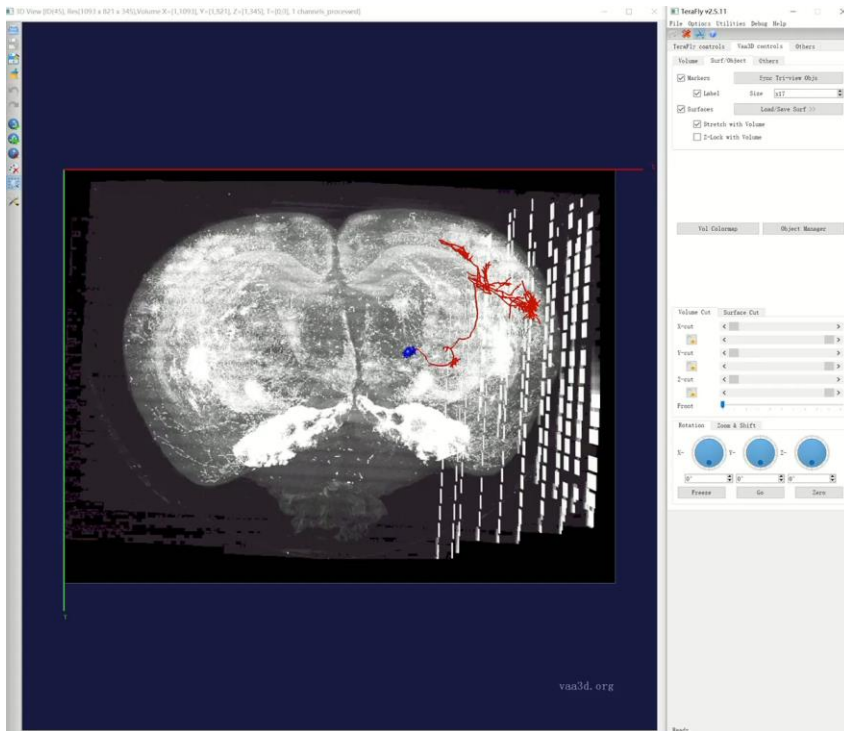


- Use VR and VR-Farm



Vaa3D-TeraFly: Post-processing by running the plugin

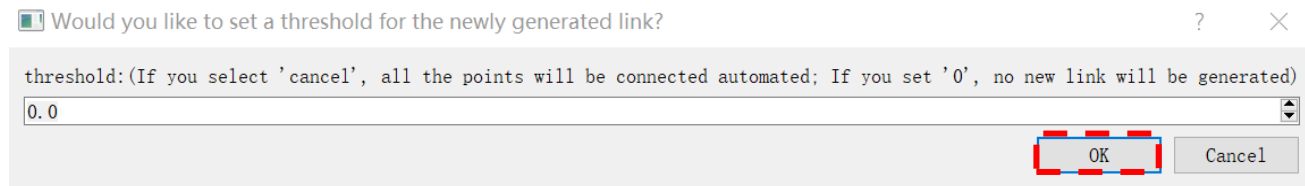
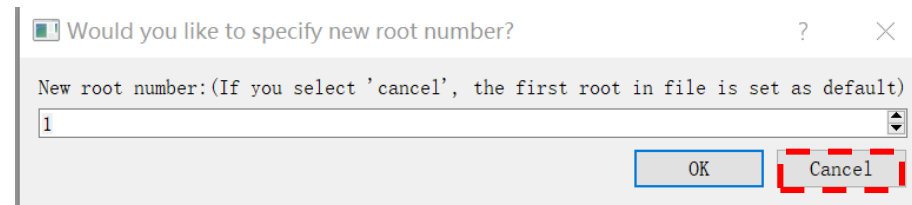
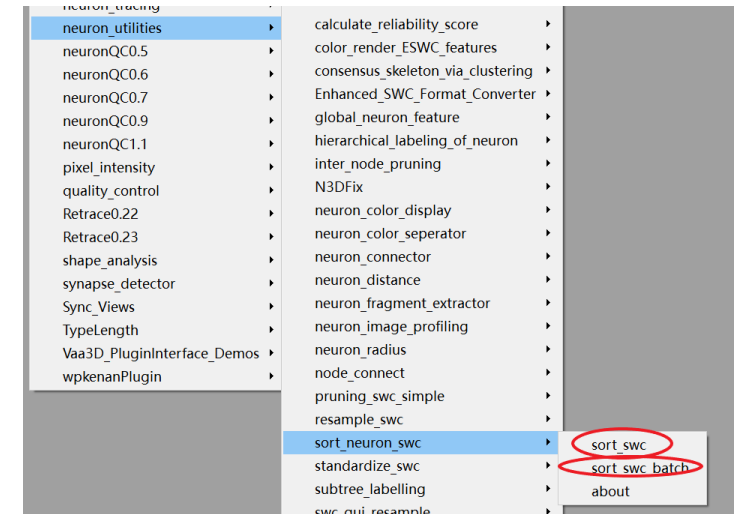
1. Soma node is defined and labeled as type '1'



2. Sort swc file:

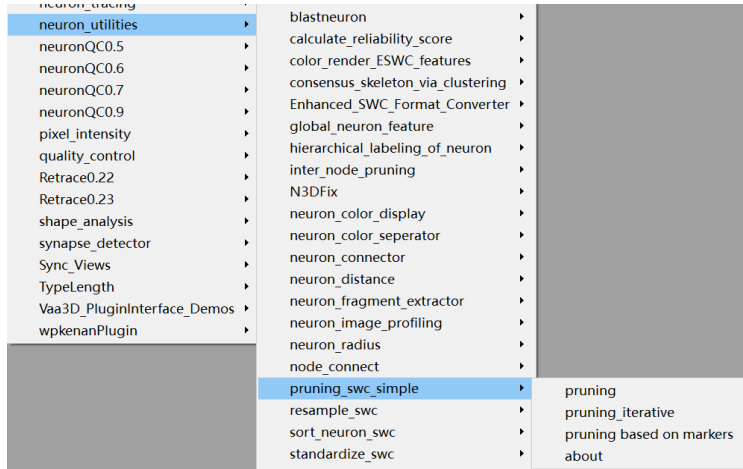
sort_swc: Process a single file;

Sort_swc_batch: Batch processing of files



3. Pruning swc file: Cut off short branches of a specific length

- Process a single file: Use pruning_swc_simple plugin



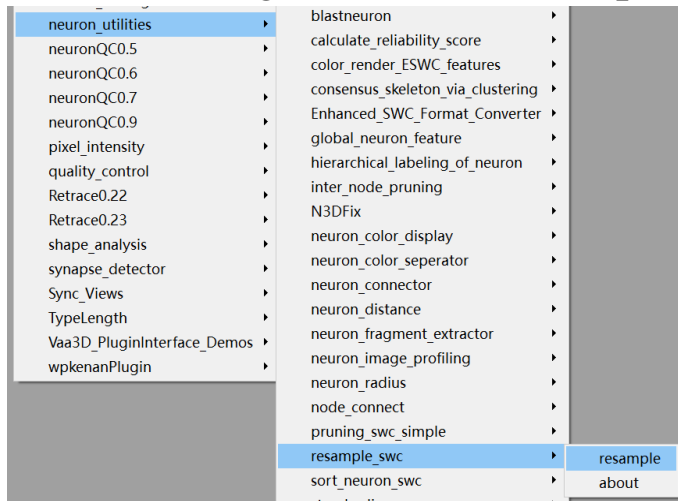
- Batch processing of files : Use python

```
import os
## swc pruning 批量操作
source="C:\Users\BrainCenter2\Desktop\20210603\20210608\100sorted_raw" # swc的文件夹 集合
dest="C:\Users\BrainCenter2\Desktop\20210603\20210608\100sort_purning"
bat_path="C:\Users\BrainCenter2\Desktop\20210603\20210608" # .bat文件地址
#
files=os.listdir(source)
for file in files:
    src=source+"/"+file
    swc_list=os.listdir(src)
    cur_dest = dest + "\\" + file
    for swc in swc_list:
        if not os.path.exists(cur_dest):
            os.mkdir(cur_dest)
        with open(os.path.join(bat_path, 'a.bat'), 'w') as OPATH:
            OPATH.writelines(["@echo off",
                               "\n",
                               "E:\VAA3D ZX\VAA3D",
                               "\vaa3d_msvc.exe /x pruning_swc_simple /f pruning_iterative /i ",
                               src+"\\"+swc+" /o ",
                               cur_dest+"\\"+swc+" /p 10"])
        filepath_Aff = os.path.join(bat_path, 'a.bat')
        os.system(filepath_Aff)
```

First box : path of swc files;
Second box: path of swc after pruning ;
Third box: bat path;
Fourth box: path of Vaa3D;
The last one: Short branch length.

4. Resample swc file: Rearrange the node distance

- Process a single file: Use resample_swc plugin



- Batch processing of files : Use python

```
import os
source = r"C:\Users\BrainCenter2\Desktop\20210603\20210608\100sorted_raw" # swc的文件夹 集合
dest = r"C:\Users\BrainCenter2\Desktop\20210603\20210608\100sort_resample"
bat_path = r"C:\Users\BrainCenter2\Desktop\20210603\20210608" # .bat文件地址
#
files = os.listdir(source)
for file in files:
    src = source + "/" + file
    swc_list = os.listdir(src)
    cur_dest = dest + "\\ " + file
    for swc in swc_list:
        if not os.path.exists(cur_dest):
            os.mkdir(cur_dest)
        with open(os.path.join(bat_path, 'a.bat'), 'w') as OPATH:
            OPATH.writelines(["@echo off",
                               "\n",
                               "E:\VAA3D_ZX\VAA3D",
                               "\\vaa3d_msvc.exe /x resample_swc /f resample_swc /i ",
                               src + "\\ " + swc + " /o ",
                               cur_dest + "\\ " + swc + " /p 4"],
                               "\n")
        filepath_Aff = os.path.join(bat_path, 'a.bat')
    os.system(filepath_Aff)
```

First box : path of swc files;
Second box: path of swc after pruning ;
Third box: bat path;
Fourth box: path of Vaa3D;
The last one: node distance.

Vaa3D-TeraFly : Quality control of neuron reconstruction

Automatic check: NeuronQC plugin

- neuronQC0.9 ▶
- neuronQC1.1 ▶**
- pixel_intensity ▶
- quality_control ▶

File Explorer view showing SWC files in a directory:

名称	修改日期	类型	大小
17781_00001.swc_sorted.swc	2021/6/4 11:04	SWC 文件	2,596 KB
17781_00003.swc_sorted.swc	2021/6/4 11:04	SWC 文件	284 KB
17781_00004.swc_sorted.swc	2021/6/4 11:04	SWC 文件	736 KB
17786_00001_U_SYY_stamp_2021_01_27_1...	2021/6/4 11:04	SWC 文件	449 KB
18047_00047.swc_sorted.swc	2021/6/4 11:04	SWC 文件	1,106 KB

Input the command: path_of_Vaa3D /x path_of_neuronQC /F neuronQC_batch /i path_of_swc /o path_of_csv /p 10 4.2 1 (Short branch length/node distance/1 refers to the loop check)

```

命令提示符
Microsoft Windows [版本 10.0.19042.1110]
(c) Microsoft Corporation。保留所有权利。

C:\Users\15951>D:\y11\y11\Vaa3D_YLL\Vaa3D_YLL\vaa3d_msvc.exe /x D:\y11\y11\Vaa3D_YLL\Vaa3D_YLL\plugins\neuronQC1.1 /f neuronQC_batch /i C:\Users\15951\Desktop\100sorted /o C:\Users\15951\Desktop\100sorted\100.csv /p 10 4.2 1
    
```

Result: 0 means unqualified; 1 means qualified.

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
neuronId	loop	loop Info	threeBifurc	threeBifurc	isSort	isSort Info	somaType	somaType	gap	gap Info	allTypes	allTypes In	shortBranc	shortBranc	nodeLengt	nodeLengt	isQualified
17781_00001	1	number of	1	0	1	continuous	1	1	1	0	1 1 2 3 4	1	0	13	0	minLength	0
17781_00003	1	number of	0	1	1	continuous	0	The first no	1	The somaT	1	The somaT	1	The somaT	1	The somaT	0
17781_00004	1	number of	0	3	1	continuous	1	1	1	0	1 1 2 3 4	1	0	0	0	minLength	0
17786_00001	1	number of	1	0	1	continuous	1	1	1	0	1 1 2 3	1	0	75	0	minLength	0
18047_00047	1	number of	1	0	1	continuous	1	1	1	0	1 1 2 3 4 typ	1	0	0	0	minLength	0

Fully qualified as shown below:

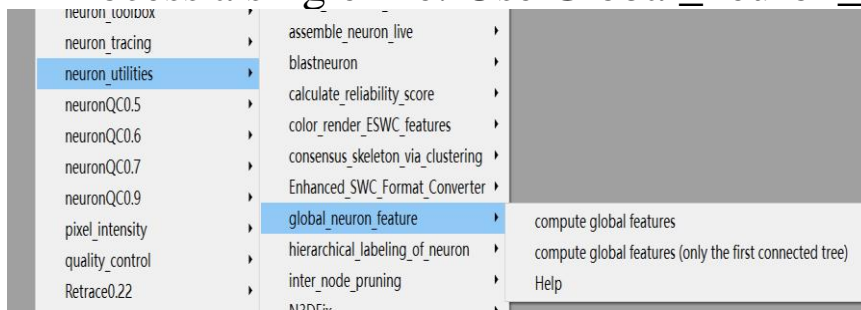
neuronId	loop	loop Info	threeBifurc	threeBifurc	isSort	isSort Info	somaType	somaType	gap	gap Info	allTypes	allTypes In	shortBranc	shortBranc	nodeLengt	nodeLengt	isQualified
18470_322	1	number of	1	0	1	continuous	1	1	1	0	1 1 2 3 4	1	0	1	1	the node le	1
18864_252	1	number of	1	0	1	continuous	1	1	1	0	1 1 2 3 4	1	0	1	1	the node le	1
18864_333	1	number of	1	0	1	continuous	1	1	1	0	1 1 2 3 4	1	0	1	1	the node le	1
18864_377	1	number of	1	0	1	continuous	1	1	1	0	1 1 2 3	1	0	1	1	the node le	1
18864_412	1	number of	1	0	1	continuous	1	1	1	0	1 1 2 3 4	1	0	1	1	the node le	1
18864_436	1	number of	1	0	1	continuous	1	1	1	0	1 1 2 3	1	0	1	1	the node le	1
18864_442	1	number of	1	0	1	continuous	1	1	1	0	1 1 2 3 4	1	0	1	1	the node le	1

Vaa3D : Analyze neuron gold standard data

- Calculate the global feature of the gold standard data of each cell type, analyze and build a model
- Quantification and analysis of neuron dendritic radius
- Quantification of soma surface
- Projection area of each cell type

Expansion 1: Global_neuron_feature plugin

- Process a single file: Use Global_neuron_feature plugin



Global features of the neuron:

```

number of nodes           : 449
soma surface              : 12.5664
number of stems           : 8
number of bifurcations    : 117
number of branches        : 240
number of tips            : 124
overall width             : 91.8282
overall height            : 133.041
overall depth             : 249.429
average diameter          : 1.99555
total length              : 2676.5
total surface             : 16816.9
total volume              : 8408.47
max euclidean distance    : 188.366
max path distance         : 372.526
max branch order          : 26
average contraction        : 0.95394
average fragmentation     : 0.866667
average parent-daughter ratio : 1
average bifurcation angle local : 64.4414
average bifurcation angle remote : 66.1306
Hausdorff dimension       : 1.34882
    
```

- Batch processing of files: input the

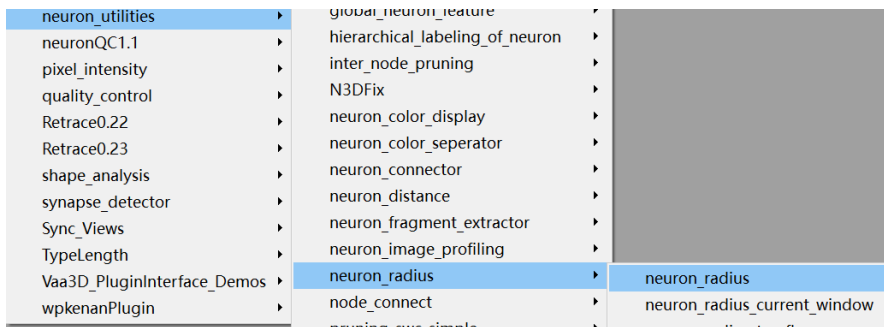
	v3d software	plugin name	function name	path of swc files	where to save
	↓	↓	↓	↓	↓
W	v3d	/x global_neuron_feature	/f compute_feature_in_folder	/i path_of_swc_folder	/o csv_to_save
L/M	v3d	-x global_neuron_feature	-f compute_feature_in_folder	-i path_of_swc_folder	-o csv_to_save

- Result:

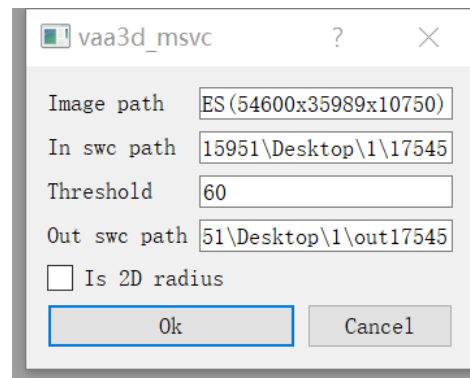
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
ID	CellType	Number of	Soma Surf	Number of	Number of	Number of	Number of	Overall Wi	Overall He	Overall De	Average D	Total Leng	Total Surfa	Total Volu	Max Euclid	Max Path L	Max Branc	Average Ci	Average Fr
r10_21025	ACAd	600	12.5664	8	207	420	215	442.072	259.207	266.175	1.99333	2975.27	18694.2	9347.1	320.955	480.154	27	0.989722	0.42381
r10_21025	ACAd	372	12.5664	6	141	286	147	291.166	196.877	224.56	1.98925	1506.77	9467.33	4733.66	125.546	304.191	18	0.987063	0.293706
r10_18047	ACAv	1084	12.5664	7	425	855	432	399.21	237.492	263.53	1.99631	4207.39	26435.8	13217.9	203.308	491.991	35	0.992452	0.265497
r10_18047	ACAv	1068	12.5664	11	414	837	425	397.839	237.676	261.272	1.99625	4210.36	26454.5	13227.2	203.48	486.969	35	0.992428	0.273596
r10_18453	ACAv	601	12.5664	8	212	431	221	374.258	154.389	282.361	1.99334	2721.07	17097	8548.49	172.329	239.192	32	0.982526	0.389791
r10_18457	ACB	212	12.5664	5	100	204	106	200.95	254.524	208.275	1.98113	385.636	2423.02	1211.51	67.6218	94.243	23	0.999418	0.029412
r10_18457	ACB	283	12.5664	6	133	270	139	221.53	249.226	192.809	1.98587	491.542	3088.45	1544.23	88.4742	145.829	33	0.999049	0.040741
r10_18457	ACB	227	12.5664	4	106	216	112	217.886	244.713	193.286	1.98238	376.359	2364.73	1182.37	78.1844	138.583	27	0.999223	0.041667
r10_18458	ACB	458	12.5664	4	213	430	219	390.784	254.249	205.995	1.99127	962.73	6049.01	3024.5	223.554	304.89	67	0.998981	0.060465
r10_18465	ACB	282	12.5664	5	136	275	141	230.741	260.133	185.252	1.98582	471.975	2965.5	1482.75	71.3951	101.803	32	0.999672	0.018182
r10_18465	ACB	253	12.5664	6	122	248	128	215.391	258.059	212.874	1.98419	415.043	2607.79	1303.9	78.6104	96.131	25	0.999868	0.012097
r10_18465	ACB	209	12.5664	7	98	201	105	213.117	258.943	199.816	1.98086	348.684	2190.85	1095.42	77.2898	108.513	27	0.999188	0.029851

Expansion 2: Neuron_radius plugin

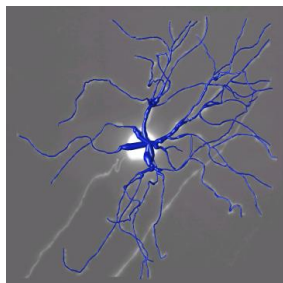
- Path:



- Enter: Image path, swc path, Threshold, out swc path, click ok.



- Radius of dendrites



```
##n type x y z radius parent
2 3 15372.3 21628.5 4359.01 1.0 3
3 3 15367.2 21631.8 4359.01 1.1 1111 4
4 3 15361.5 21634.0 4358.8 1.14815 5
5 3 15358.7 21633.4 4358.64 1.23395 6
6 3 15352.8 21634.7 4358.43 1.44444 7
7 3 15346.7 21636.7 4358.43 1.62414 8
8 3 15341.8 21640.8 4358.43 1.47965 9
```



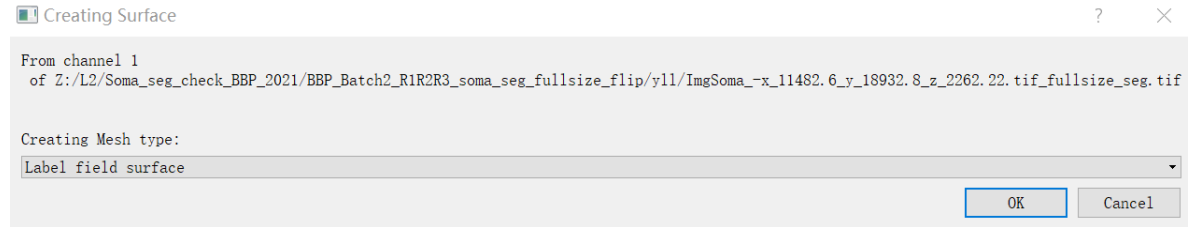
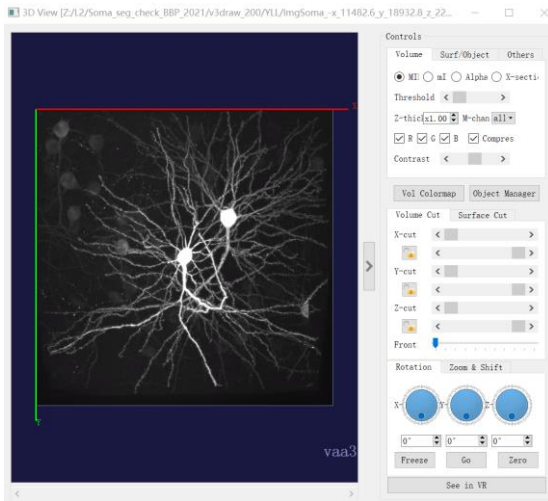
The neuron node has row index 1502

- 1) node # = 1503
- 2) type = 3
- 3) x coord = 15063.4
- 4) y coord = 21893.1
- 5) z coord = 4364
- 6) radius = 1.2
- 7) parent = 1504
- segment (index) = -1 (0)

- Check: Drag in the original image, Determine whether the generated radius is consistent with the image.

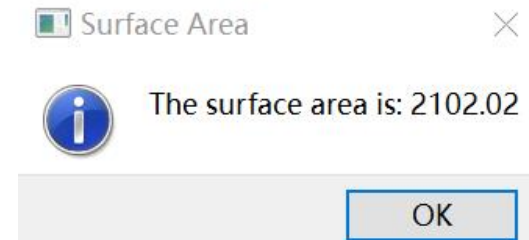
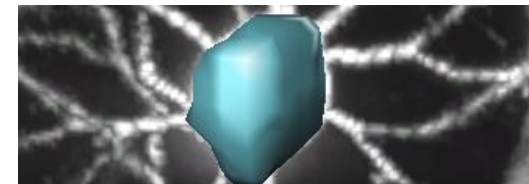
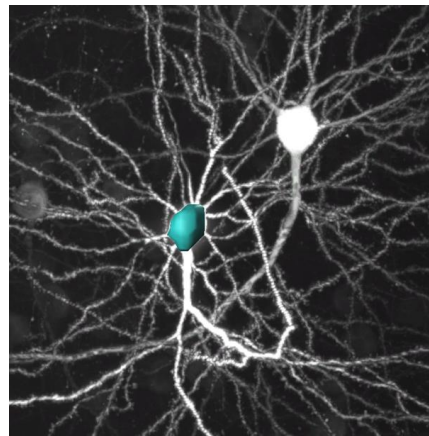
Expansion 3: Soma surface

- Drag the V3Draw file into Vaa3D, see in 3D.
- Drag the TIF file into 3D image, creating mesh type, choose 'Label field surface'.



- Creating Mesh method: Marching Cubes
- Creating Mesh density: 100
- No, Yes.

- Result: Create the surface, and you can quantify the surface area.





Thank you!