

Quick manual for cell analysis module of CurveAlign+

(Updated on May 19,2022)

This CurveAlign cell analysis module segments cells using deep learning based models including [StarDist](#), [Cellpose](#) and [DeepCell](#) from H&E bright field image/ fluorescence image/ phase contrast image. The segmented cells are then quantified and used in density based tumor area annotation. The fibers extracted from CurveAlign can be imported into this module and can be quantified with respect to each tumor region. The cells in each annotated tumor region can also be quantified.

1 Installation

1.1 Third-party tools

1. StarDist

<https://github.com/stardist>

2. Cellpose

<https://github.com/mouseland/cellpose>

3. DeepCell

<https://github.com/vanvalenlab/deepcell-tf>

4. Vampire

https://github.com/kukionfr/VAMPIRE_open

1.2 Launch from MATLAB (source-code version)

1.2.1 Set up python environment

```
% check current python environment  
pyenv  
%terminate current environment  
terminate(pyenv)  
%set new pyenv
```

```
pyenv('Version','/Users/ympro/opt/anaconda3/envs/SDpy38/bin/python', 'ExecutionMode',  
'OutOfProcess')
```

Example of PythonEnvironment with properties:

Version: "3.8"

Executable: "/Users/ympro/opt/anaconda3/envs/SDpy38/bin/python"

Library: "/Users/ympro/opt/anaconda3/envs/SDpy38/lib/libpython3.8.dylib"

Home: "/Users/ympro/opt/anaconda3/envs/SDpy38"

Status: Loaded

ExecutionMode: OutOfProcess

ProcessID: "79433"

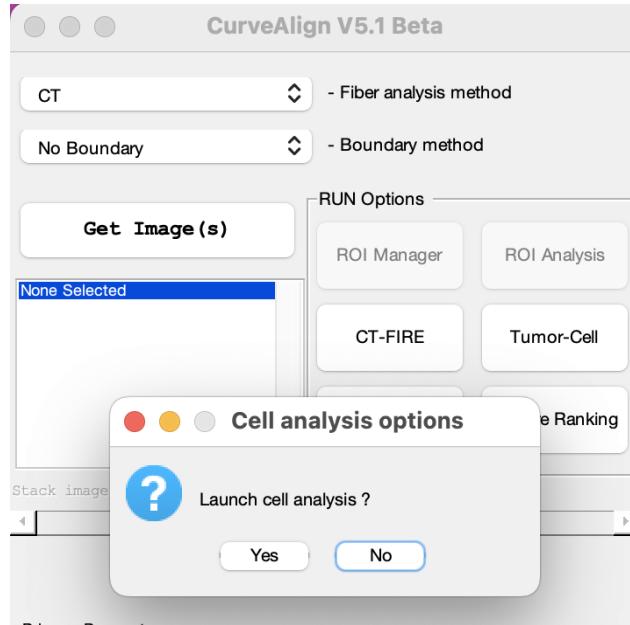
ProcessName: "MATLABPyHost"

In this conda environment “SDpy38”, StarDist, Cellpose and Vampire were installed

1.2.2 Open the mlapp file

Double click the file named “CellAnalysisForCurveAlign.mlapp” under the */curvelets/Cellanalysis/ folder. Then click the Green “Run” button to launch the APP.

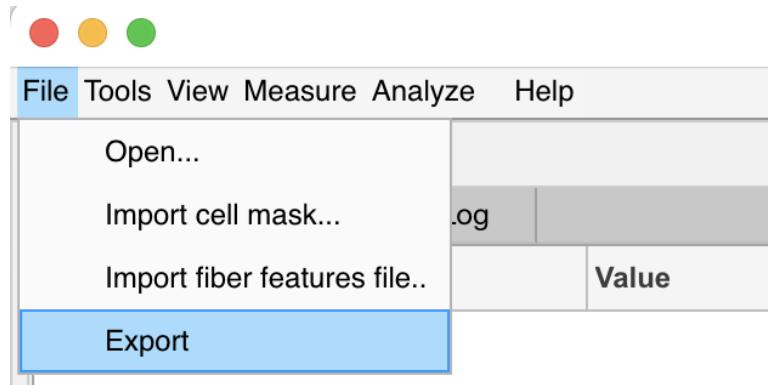
Alternatively, launch this cell analysis module from CurveAlign by clicking the “Tumor-Cell” button and the choose “Yes” as displayed below.



To be noted, the mlapp APP is implemented in MATLAB 2021a. If it is opened with versions earlier than 2021a, one or more functionalities might not be available.

1.3 Export results

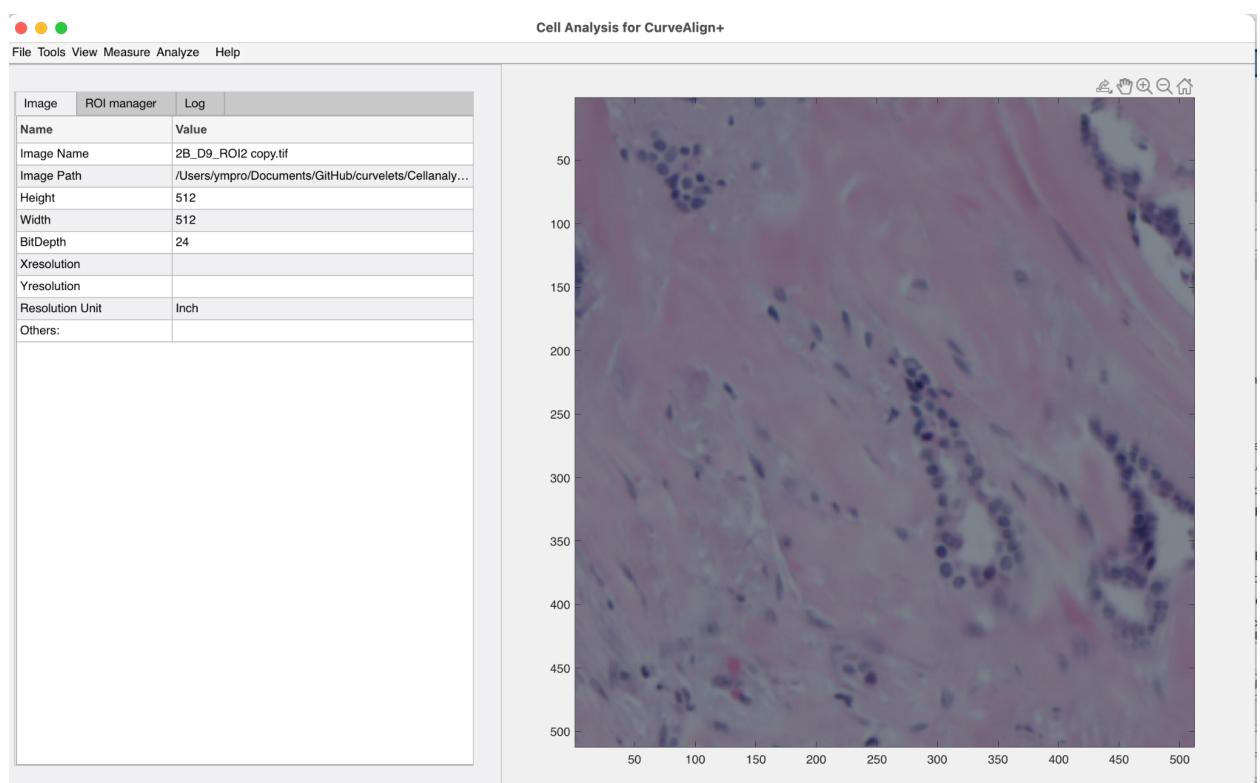
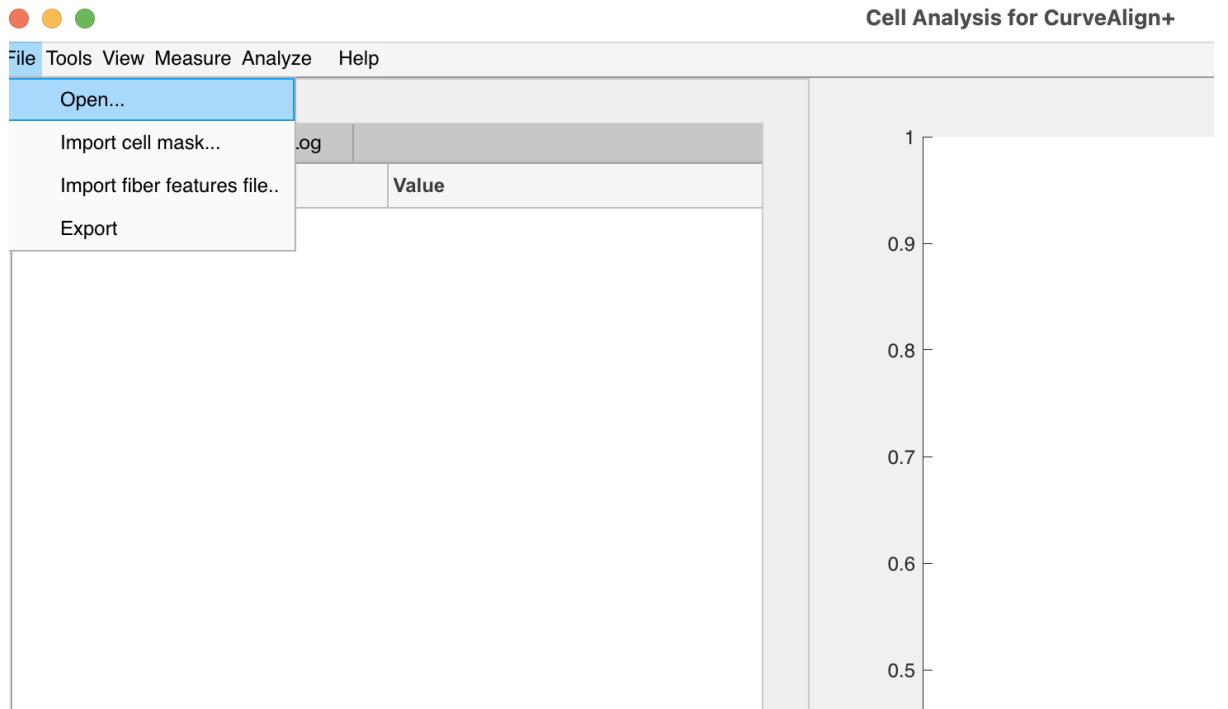
Click the “File->Export” to automatically save the cell and tumor information.



2 Tutorials

Tutorial 1: StarDist for HE bright field images

Step 1: Open the HE bright field image named “”



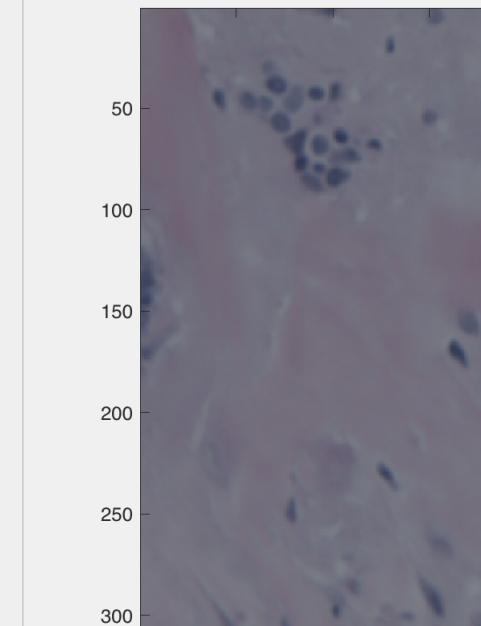
Step 2: Click Analyze->Cell Analysis to open “Cell Analysis” parameters setting window.



Cell Analysis for CurveAlign+

File Tools View Measure Analyze Help

	Preprocessing	
Image	ROI manager	Cell Analysis
Name		Tumor region annotation
Image Name		Fiber quantification
Image Path		TACSSs calculation
Height	512	Hub/curvelets/Cellanaly...
Width	512	
BitDepth	24	
Xresolution		
Yresolution		
Resolution Unit	Inch	
Others:		





Cell Analysis-options

Path to image

/Users/ympro/Documents/GitHub/curvelets/Ce

Image type

HE bright field

Object type

Nuclei

Methods

StarDist

Pre-trained models

2D_versatile_he

Default parameters

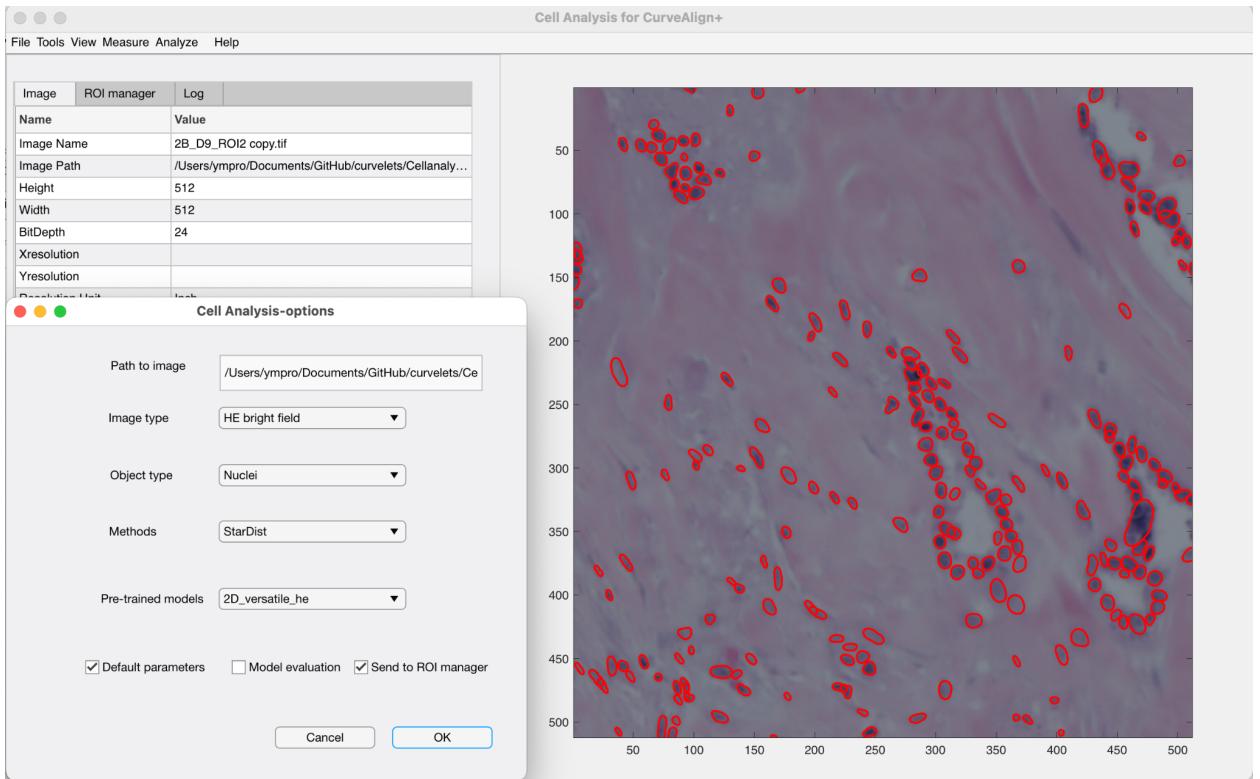
Model evaluation

Send to ROI manager

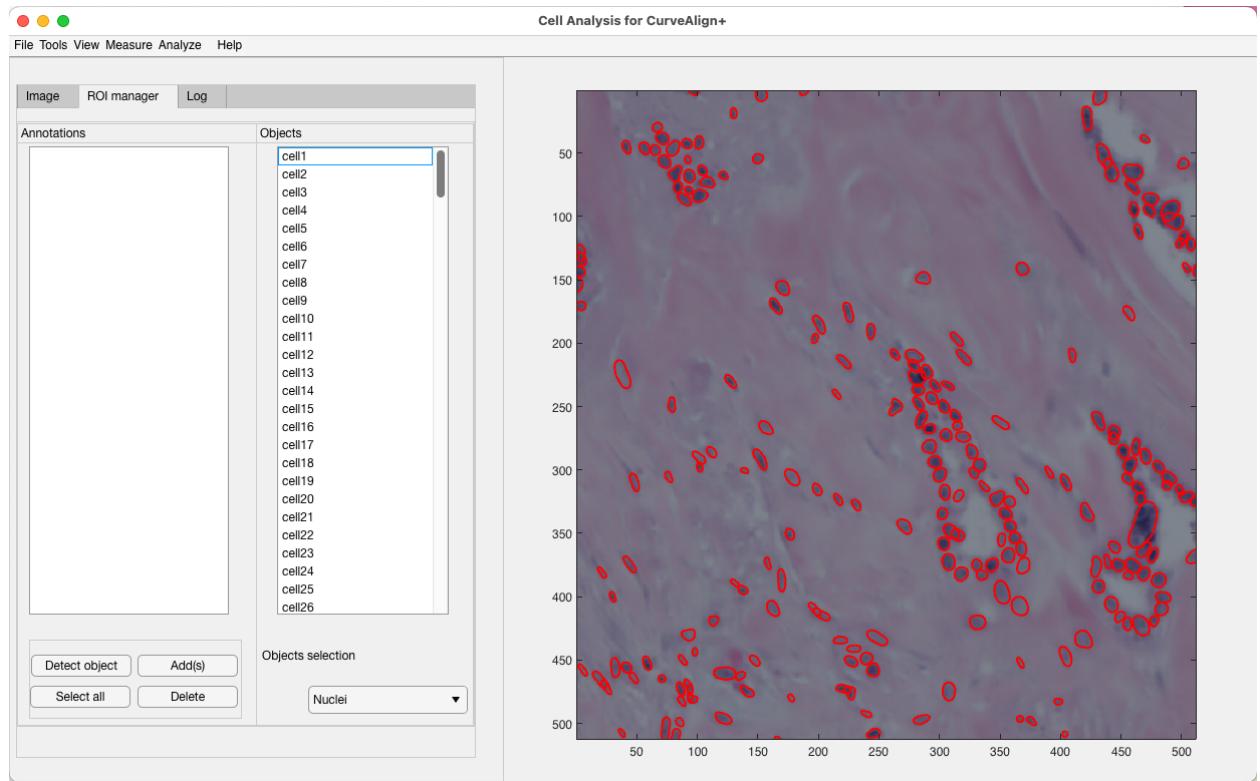
Cancel

OK

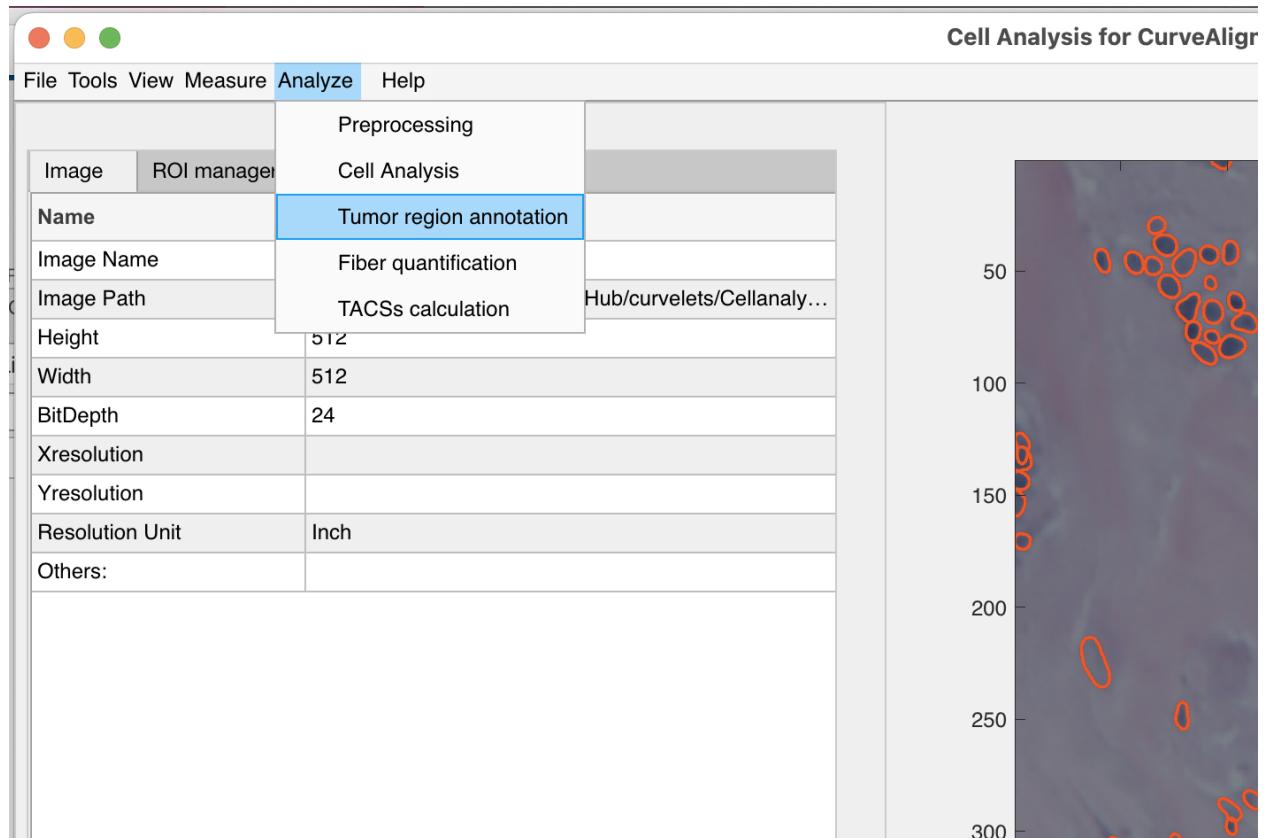
Step 3: Click “OK” to use the default settings to run the cell segmentation



Step 4: Click “Cancel” button to close the Window and click the “ROI manager” tab in the main GUI to check the segmented cells.



Step 5: Click Analyze->Tumor region annotation to open the density based tumor annotation module.



Tumor Region Detection-options

Path to image /Users/ympro/Documents/GitHub/curvelets/Cellanaly...

Image type HE bright field

Annotation Methods Ranking

Grid Columns 50

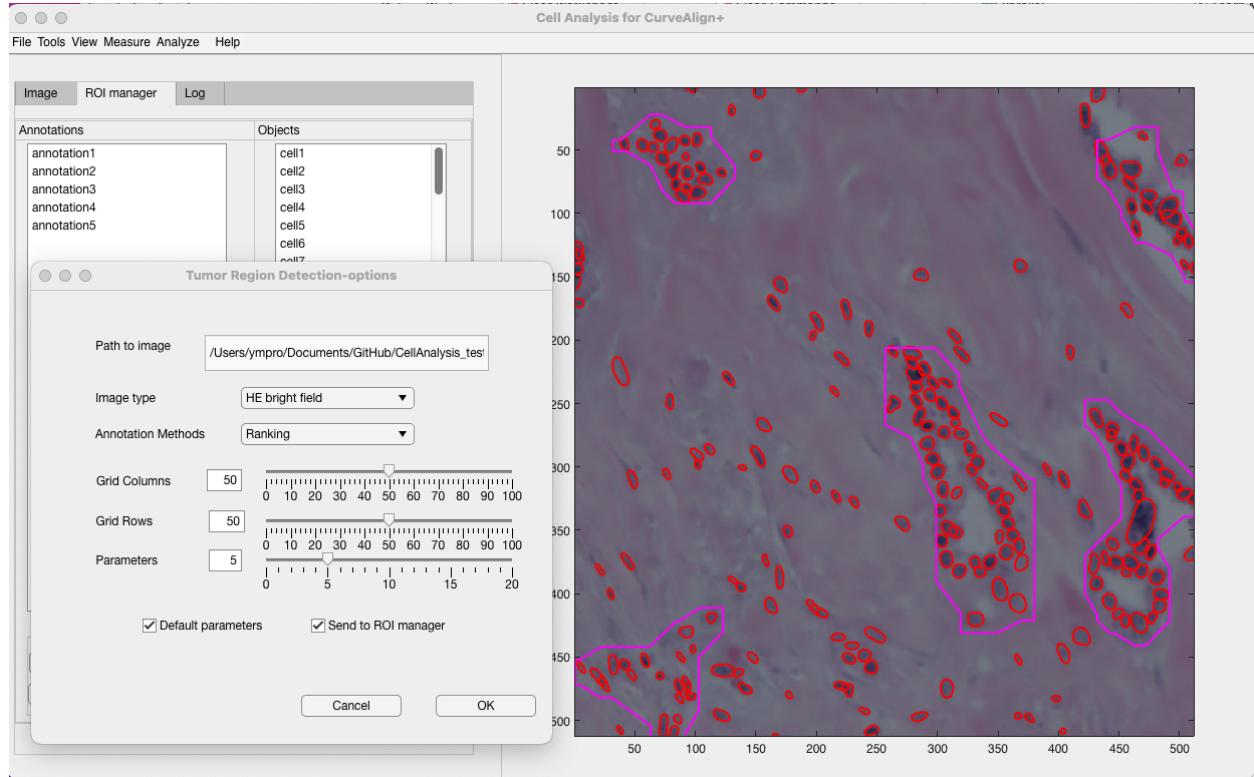
Grid Rows 50

Parameters 5

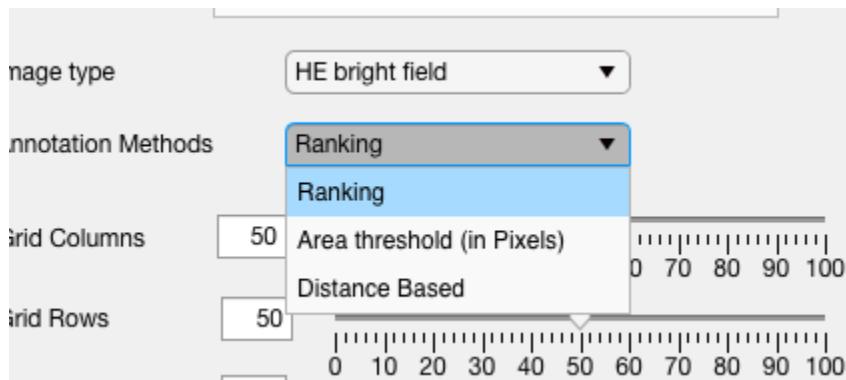
Default parameters Send to ROI manager

Cancel OK

Step 6: Click the “OK” button to use default parameters to get the annotations.

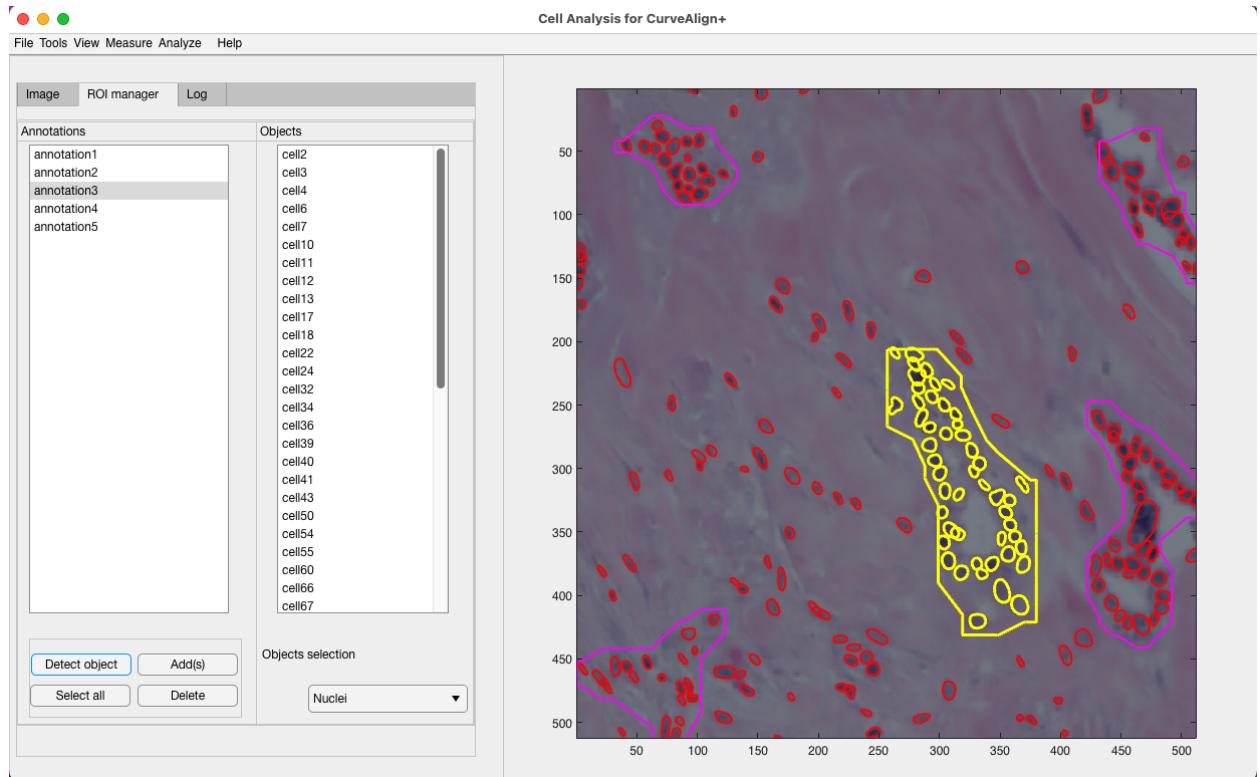


Other two methods are also available as shown below:



To be noted, currently this automatic annotation only works for images processed by “StarDist”

Step 7: After clicking the “Cancel” button to close the tumor annotation options window, select the “annotation3” in the annotation list, click the “Detect object” button to get the cells within the annotation3. Selected annotation and cells are highlighted in “yellow” color as shown below.



Step 8: Show the object measurements by clicking “Measure->Show object measurements”.

Object measurements

Image	Name	Class	Parent	Center-X	Center-Y	Orientation	Area	Circularity	Shape
2B_D9_ROI2.tif	cell2	Cell	Tumor-annotation3	318.0000	382.0000	42.9073	375.0000	1.0722	
2B_D9_ROI2.tif	cell3	Cell	Tumor-annotation3	304.0000	358.0000	114.8413	303.0000	1.0733	
2B_D9_ROI2.tif	cell4	Cell	Tumor-annotation3	307.0000	372.0000	99.4591	429.0000	1.0588	
2B_D9_ROI2.tif	cell6	Cell	Tumor-annotation3	279.0000	217.0000	129.0893	263.0000	0.9858	
2B_D9_ROI2.tif	cell7	Cell	Tumor-annotation3	333.0000	296.0000	128.1994	313.0000	1.0818	
2B_D9_ROI2.tif	cell10	Cell	Tumor-annotation3	304.0000	250.0000	123.9755	270.0000	1.0401	
2B_D9_ROI2.tif	cell11	Cell	Tumor-annotation3	354.0000	334.0000	145.8990	283.0000	1.0761	
2B_D9_ROI2.tif	cell12	Cell	Tumor-annotation3	357.0000	367.0000	76.0016	389.0000	0.9936	
2B_D9_ROI2.tif	cell13	Cell	Tumor-annotation3	294.0000	244.0000	133.0829	276.0000	1.0390	
2B_D9_ROI2.tif	cell17	Cell	Tumor-annotation3	296.0000	294.0000	167.3822	310.0000	1.0791	
2B_D9_ROI2.tif	cell18	Cell	Tumor-annotation3	300.0000	304.0000	49.3048	349.0000	1.0387	
2B_D9_ROI2.tif	cell22	Cell	Tumor-annotation3	327.0000	286.0000	110.4503	314.0000	1.0240	

Histograms Close Save

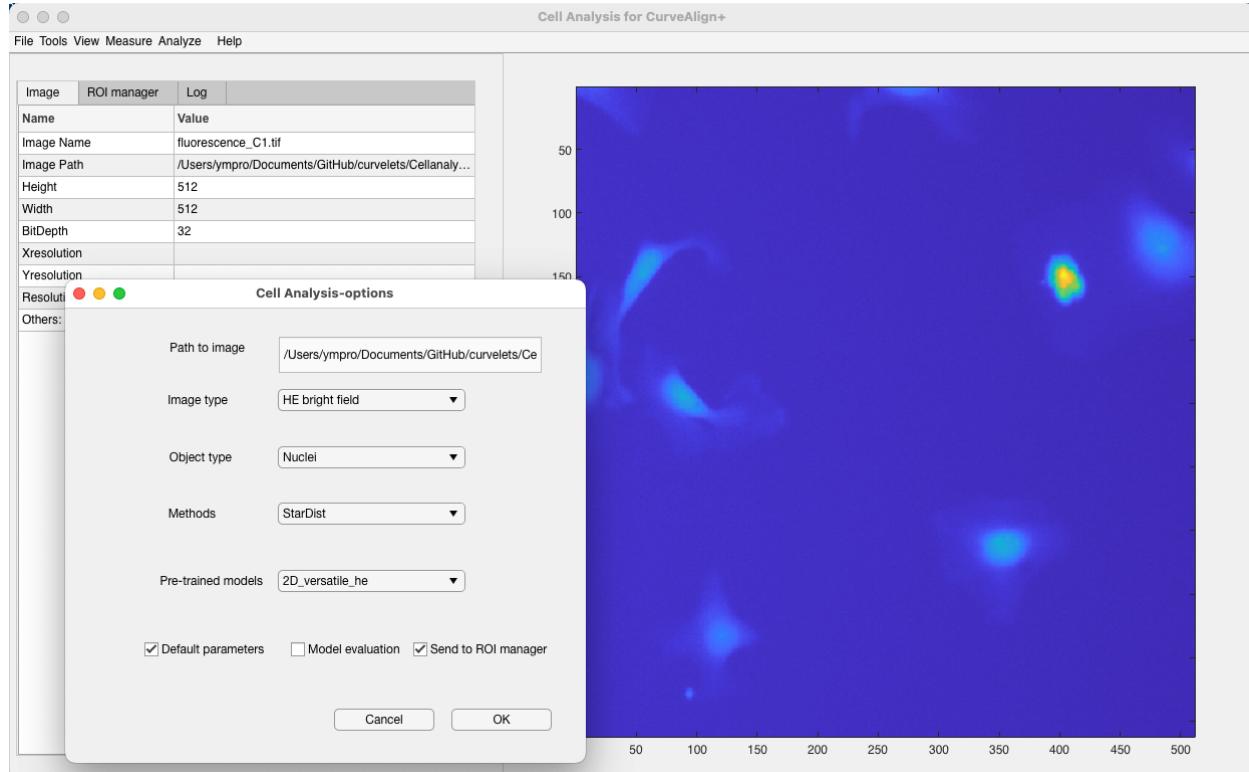
Step 9: Show the annotation measurements by clicking “Measure->Show annotation measurements”

Annotation measurements														
Image	Name	Class	Parent	Center-X	Center-Y	Tumor-Area	Tumor-Perimeter	Cell-Number	Cell-Area	Cell-Orientation	Cell-Alignment	Fiber-Number	Fiber-Orientation	
2B_D9_ROI2.tif	annotation1	Tumor	Image	324	322	15900	591	49	14853	127.3	0.3140	0	NaN	

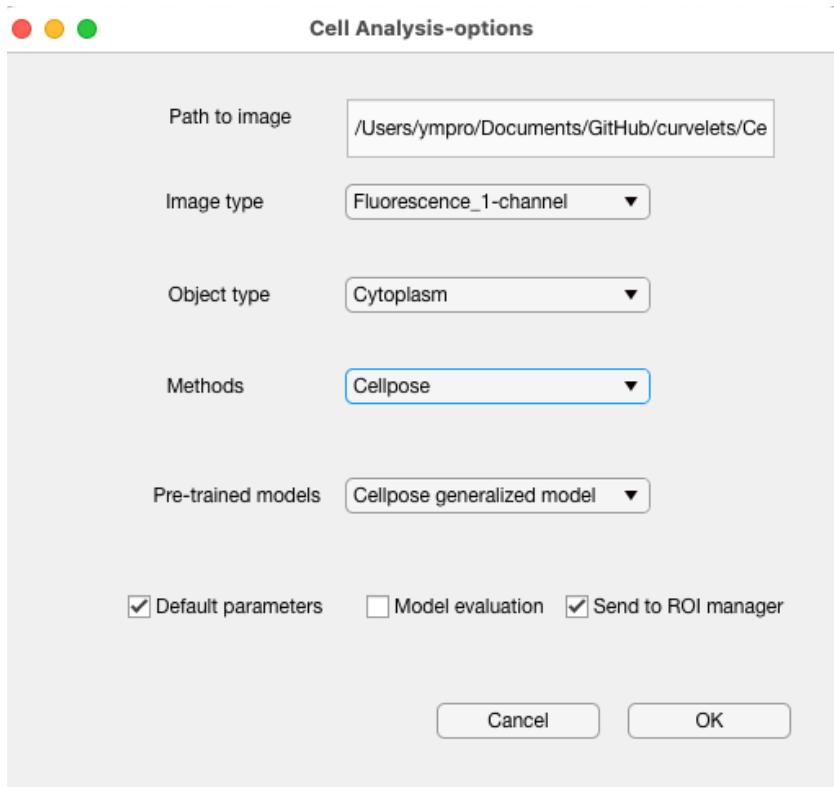
Histograms Close Save

Tutorial 2: Cellpose for fluorescence images

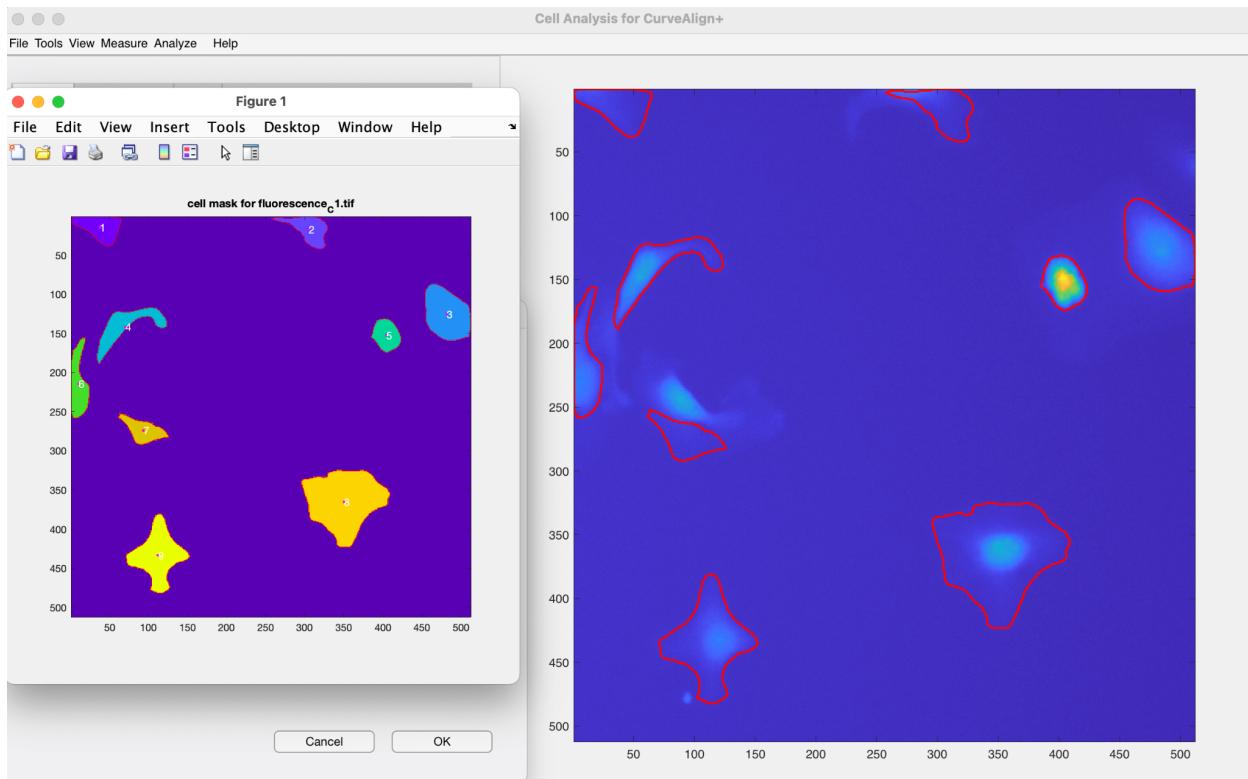
Step 1: Open the fluorescence image named “fluorescence_C1.tif” and cell analysis parameters setting window following the method described in steps 1 and 2 in tutorial 1.



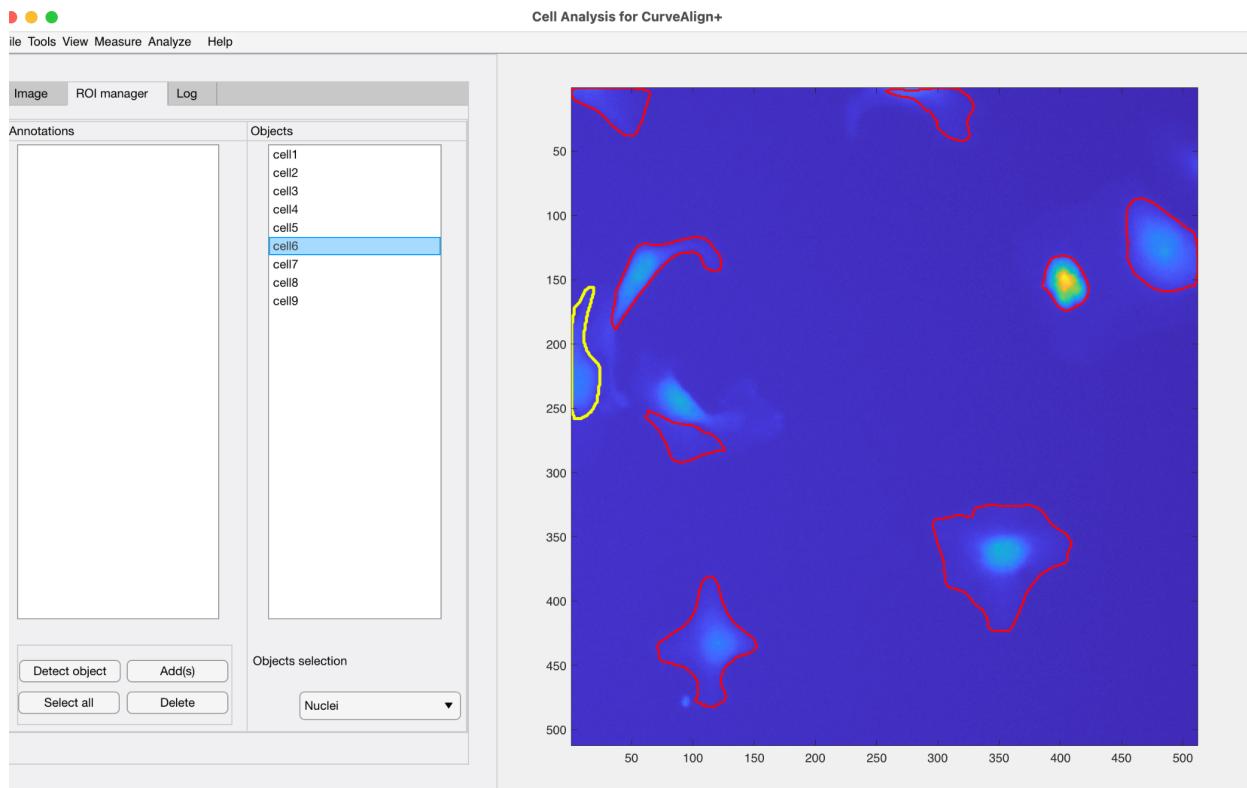
Step 2: change the image type, object type and method as shown below.



Step 3: Click the “OK” button to run Cellpose cell segmentation



Step 4: Click the “ROI manager” tab and click the “cell6” in the objects list to highlight the edges of cell6 in yellow.



Step 5: Show the object measurements by clicking “Measure->Show object measurements”.

Object measurements									
Image	Name	Class	Parent	Center-X	Center-Y	Orientation	Area	Circularity	Shape
fluorescence_C1.tif	cell1	cell	TumorAnnotation	37.4684	14.7309	-16.6601	1535	0.6974	
fluorescence_C1.tif	cell2	cell	TumorAnnotation	304.9068	17.0735	-28.0731	1470	0.5073	
fluorescence_C1.tif	cell3	cell	TumorAnnotation	481.7548	125.3027	-56.2894	3307	0.9031	
fluorescence_C1.tif	cell4	cell	TumorAnnotation	70.4039	141.9376	29.7482	2050	0.3391	
fluorescence_C1.tif	cell5	cell	TumorAnnotation	404.4526	152.3931	-64.6485	1193	0.9800	
fluorescence_C1.tif	cell6	cell	TumorAnnotation	10.3580	214.3635	-88.9276	1634	0.3947	
fluorescence_C1.tif	cell7	cell	TumorAnnotation	93.1934	274.0112	-23.0447	1246	0.5821	
fluorescence_C1.tif	cell8	cell	TumorAnnotation	350.2230	365.4350	9.3054	6988	0.7557	
fluorescence_C1.tif	cell9	cell	TumorAnnotation	111.7743	433.6030	84.5591	3708	0.5494	

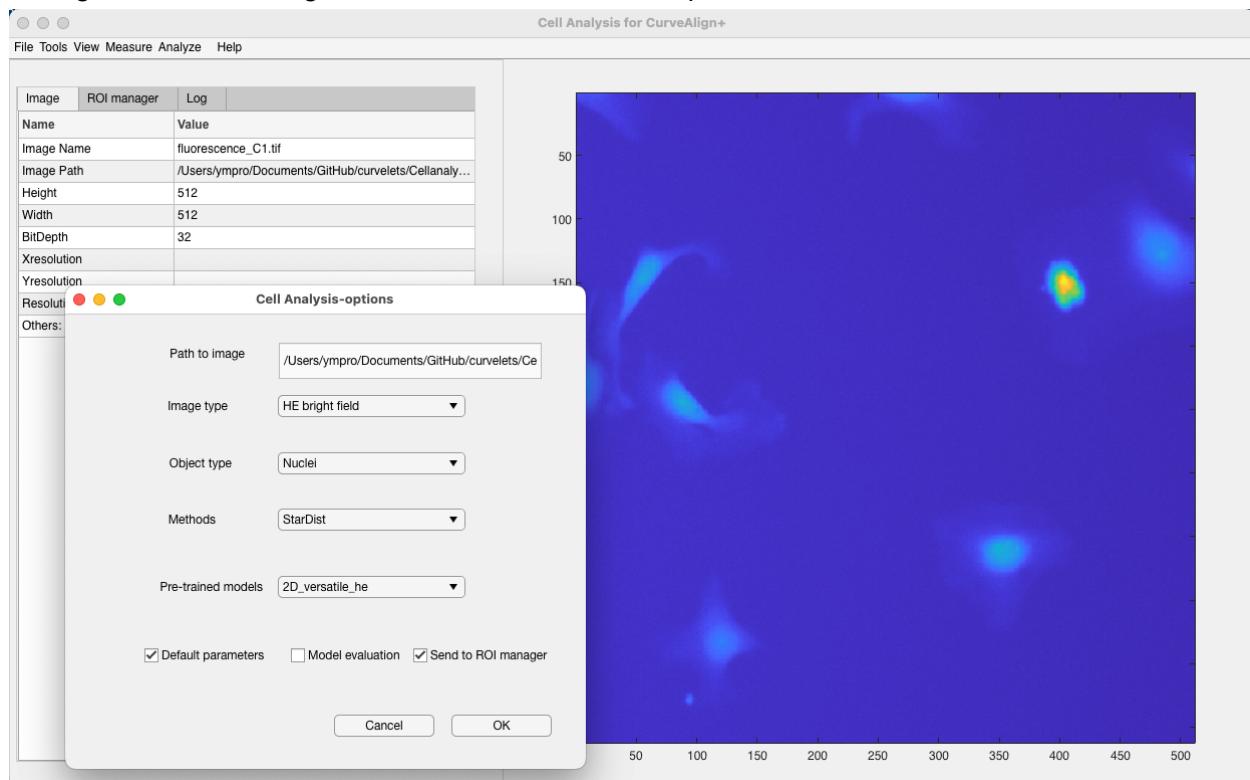
Tutorial 3: DeepCell for fluorescence images

Step 0: make sure there is a correct python environment for deepcell.

If needed, terminate current pyenv and change the pyenv to DeepCell directory following the steps in 1.2.1. An example is shown below:

```
%terminate current environment  
terminate(pyenv)  
%set new pyenv  
pyenv('Version','/Users/ympro/opt/anaconda3/envs/deepcell/bin/python', 'ExecutionMode',  
'OutOfProcess')  
reboot MATLAB
```

Step 1: Open the fluorescence image named “fluorescence_C1.tif” and cell analysis parameters setting window following the method described in steps 1 and 2 in tutorial 1.



Step 2: change the image type, object type and method as shown below.



Cell Analysis-options

Path to image

Image type

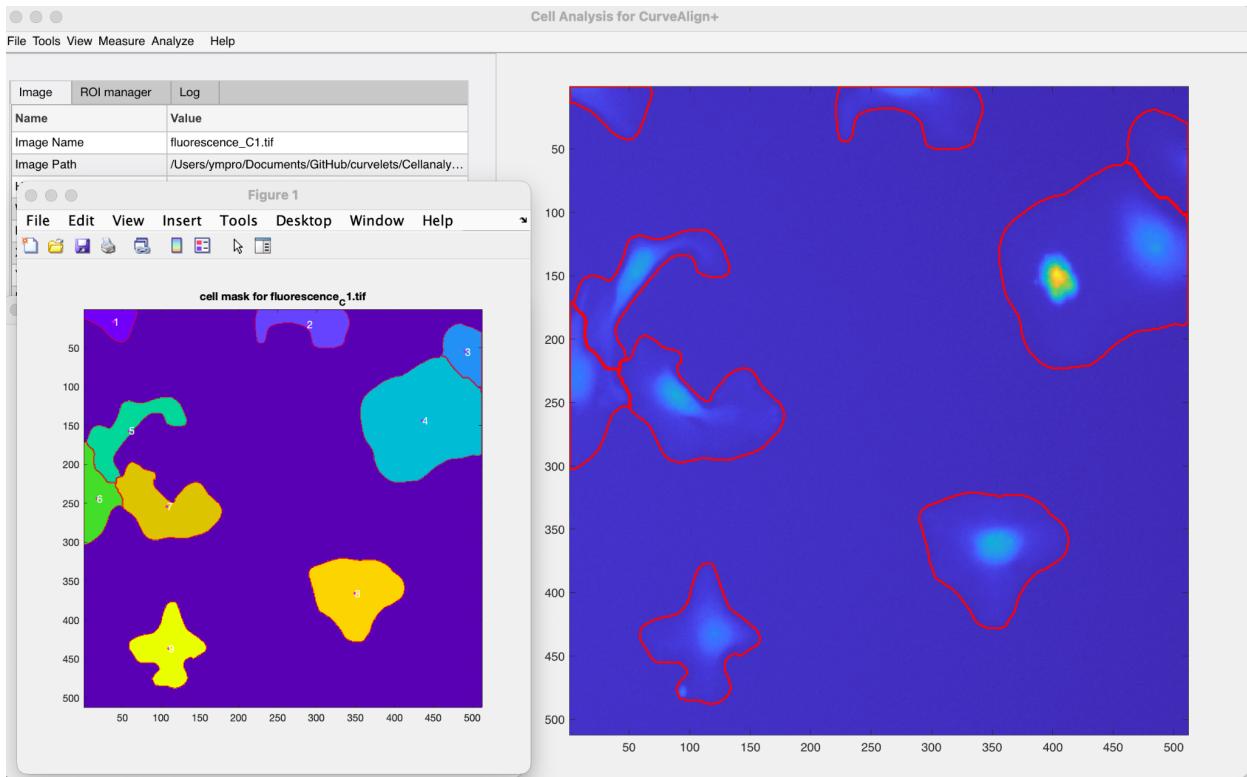
Object type

Methods

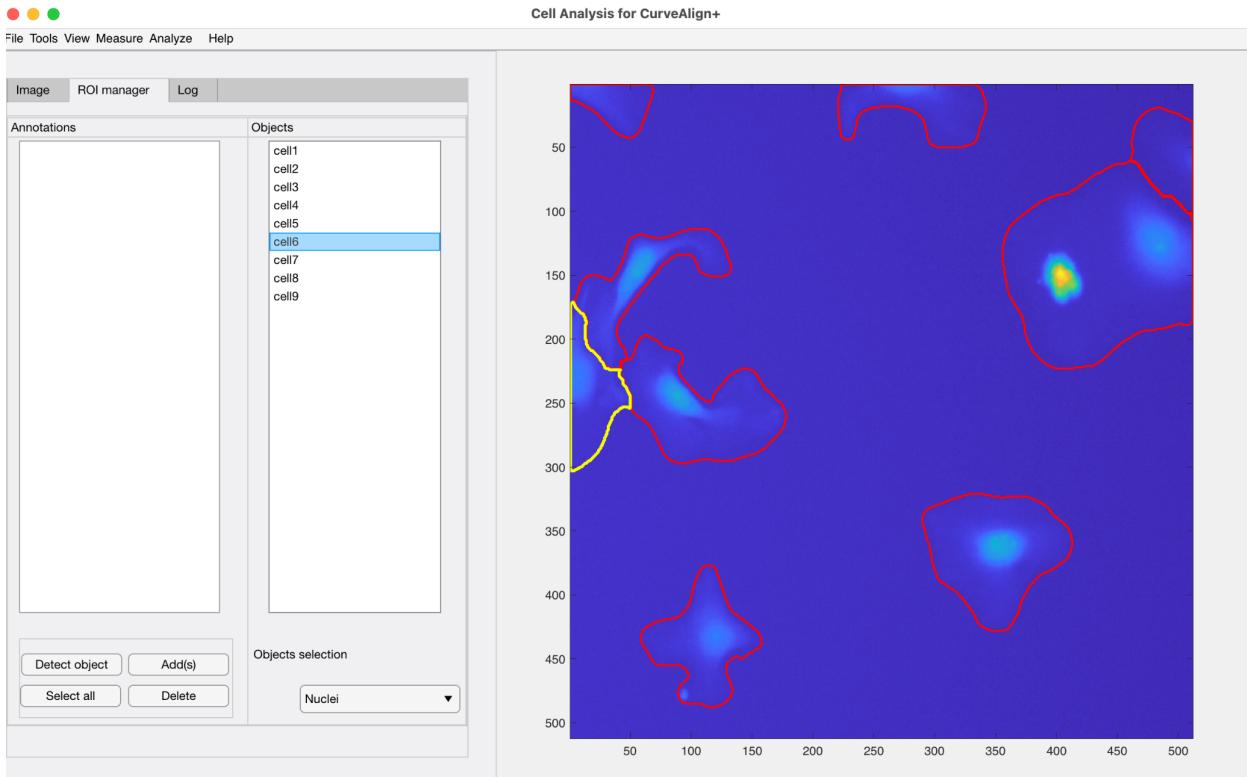
Pre-trained models

Default parameters Model evaluation Send to ROI manager

Step 3: Click the “OK” button to run DeepCell cell segmentation.



Step 4: Click the “ROI manager” tab and click the “cell6” in the objects list to highlight the edges of cell6 in yellow.



Step 5: Show the object measurements by clicking “Measure->Show object measurements”.

The screenshot shows a software window titled "Object measurements". At the top, there are three colored circles (red, yellow, green) and a title bar. Below the title bar is a table with the following data:

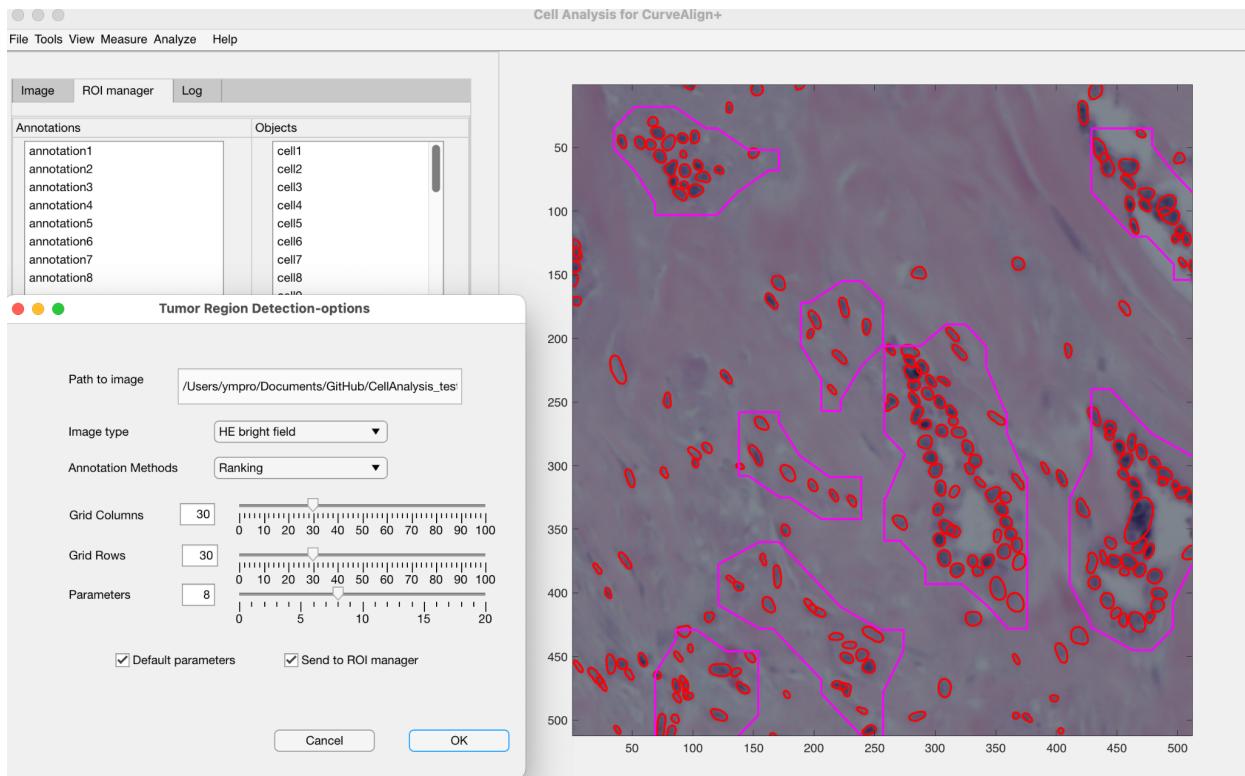
Image	Name	Class	Parent	Center-X	Center-Y	Orientation	Area	Circularity	Shape
fluorescence_C1.tif	cell1	cell	TumorAnnotation	38.6345	16.6262	-14.9001	1937	0.7204	
fluorescence_C1.tif	cell2	cell	TumorAnnotation	287.7030	20.1618	-5.4316	4060	0.4379	
fluorescence_C1.tif	cell3	cell	TumorAnnotation	490.2721	55.1265	-70.1140	3043	0.7289	
fluorescence_C1.tif	cell4	cell	TumorAnnotation	435.7518	143.7654	32.4815	18597	0.8863	
fluorescence_C1.tif	cell5	cell	TumorAnnotation	58.6808	156.8562	36.4251	4953	0.3431	
fluorescence_C1.tif	cell6	cell	TumorAnnotation	17.4503	243.9353	-87.1430	3542	0.4564	
fluorescence_C1.tif	cell7	cell	TumorAnnotation	107.2352	254.2114	-20.7880	7939	0.5880	
fluorescence_C1.tif	cell8	cell	TumorAnnotation	349.0296	365.8114	-2.3419	8788	0.8490	
fluorescence_C1.tif	cell9	cell	TumorAnnotation	109.5024	436.8472	83.4506	5518	0.5624	

At the bottom of the window are three buttons: "Histograms", "Close", and "Save".

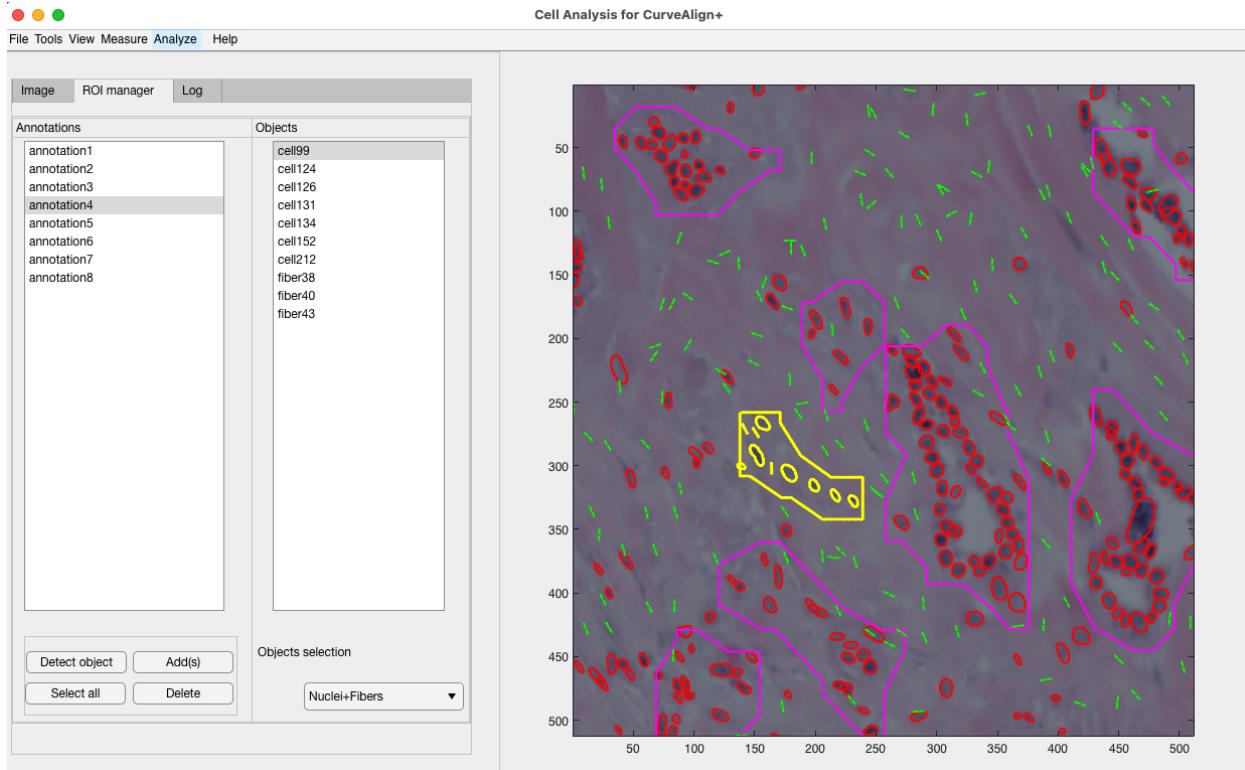
Tutorial 4: Measure both cell and fiber information in a given annotation

This function currently only works on the H&E bright field image. The fiber features are extracted from the CurveAlign and saved as .mat file. The fiber image and the H&E bright field image.

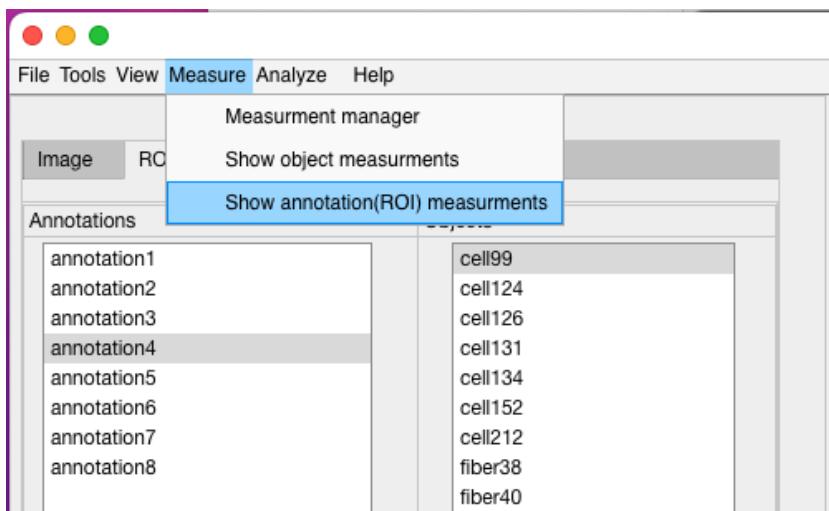
Step 1: Follow the steps 1-5 to open the tumor annotation window and set the parameters as below to get the 8 tumor regions



Step 2: Select the “annotation4” and then click the button “Detect object” to detect the cells and fibers within the annotation 4 as shown below.



Step 3: Show the measurement of the selected annotation by clicking “Measurement->Show annotation(ROI) measurements”



The summary statistics about the cells/fibers within the annotation are shown in the following table.

The screenshot shows the 'Annotation measurements' dialog box. At the top, it displays the title 'Annotation measurements'. Below the title is a table with the following data:

Image	Name	Class	Parent	Center-X	Center-Y	Tumor-Area	Tumor-Perimeter	Ce
2B_D9_ROI2.tif	annotation4	Tumor	Image	183	304	4439	313	

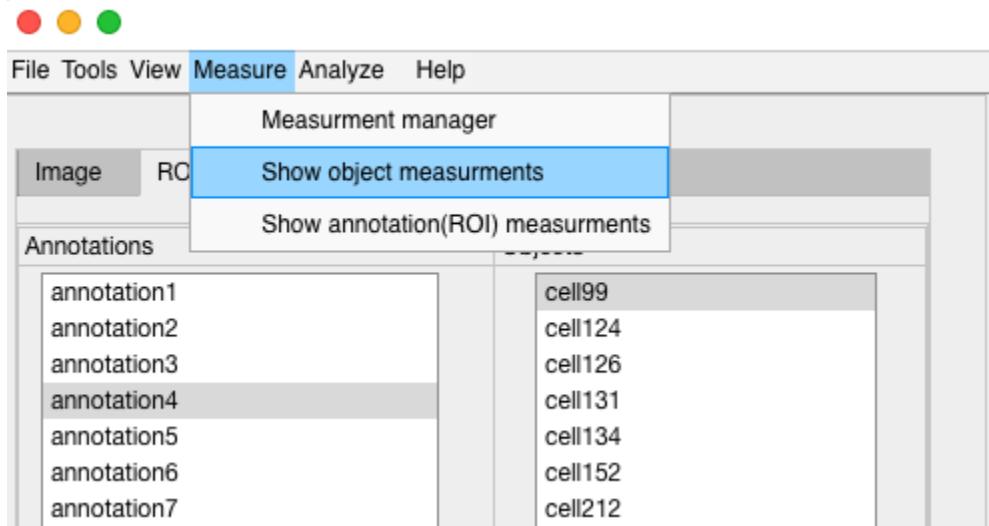
At the bottom of the dialog box, there are three buttons: 'Histograms', 'Close', and 'Save'.

Annotation measurements

	Tumor-Area	Tumor-Perimeter	Cell-Number	Cell-Area	Cell-Orientation	Cell-Alignment	Fiber-Number	Fiber-Orientation	Fiber-Alignment
4439	313	7	1790	131.0		0.8971	3	111.8	0.8810

[Histograms](#)
 [Close](#)
 [Save](#)

Step 4: Show the measurement of the selected annotation by clicking “Measurement->Show object measurements”



The measurements of the individual cells/fibers are displayed in the following table.

Object measurements

Image	Name	Class	Parent	Center-X	Center-Y	Orientation	Area	Circularity	ShapeMode
2B_D9_ROI2.tif	cell99	Cell	Tumor-annotation4	199.0000	315.0000	122.9366	208.0000	0.9993	7.0000
2B_D9_ROI2.tif	cell124	Cell	Tumor-annotation4	178.0000	306.0000	130.7596	437.0000	0.9751	1.0000
2B_D9_ROI2.tif	cell126	Cell	Tumor-annotation4	232.0000	328.0000	123.4187	174.0000	0.9895	2.0000
2B_D9_ROI2.tif	cell131	Cell	Tumor-annotation4	217.0000	324.0000	124.4957	163.0000	0.9185	3.0000
2B_D9_ROI2.tif	cell134	Cell	Tumor-annotation4	153.0000	293.0000	120.1868	397.0000	0.7602	1.0000
2B_D9_ROI2.tif	cell152	Cell	Tumor-annotation4	156.0000	266.0000	138.9840	329.0000	0.9593	1.0000
2B_D9_ROI2.tif	cell212	Cell	Tumor-annotation4	139.0000	300.0000	161.3865	82.0000	1.0466	9.0000
2B_D9_ROI2.tif	fiber38	fiber	Tumor-annotation4	151.0000	274.0000	123.6901			
2B_D9_ROI2.tif	fiber40	fiber	Tumor-annotation4	142.0000	271.0000	118.9264			
2B_D9_ROI2.tif	fiber43	fiber	Tumor-annotation4	164.0000	302.0000	91.4688			

Histograms Close Save

3 Troubleshooting

5.11.2022-DeepCell

A local file was found, but it seems to be incomplete or outdated because the auto file hash does not match the original value of 6a244f561b4d37169cb1a58b6029910f so we will re-download the data.

Downloading data from

<https://deepcell-data.s3-us-west-1.amazonaws.com/saved-models/CytoplasmSegmentation-3.tar.gz>

Warning: the font "Times" is not available, so "Lucida Bright" has been substituted, but may have unexpected appearance or behavior. Re-enable the "Times" font to remove this warning.

Warning: the font "Times" is not available, so "Lucida Bright" has been substituted, but may have unexpected appearance or behavior. Re-enable the "Times" font to remove this warning.

>> imgCardWholeCell('DeepCell','0_00 copy.tif')

A local file was found, but it seems to be incomplete or outdated because the auto file hash does not match the original value of 6a244f561b4d37169cb1a58b6029910f so we will re-download the data.

Downloading data from

<https://deepcell-data.s3-us-west-1.amazonaws.com/saved-models/CytoplasmSegmentation-3.tar.gz>

95264768/95263450 [=====] - 22s 0us/step

WARNING:tensorflow:SavedModel saved prior to TF 2.5 detected when loading Keras model.
Please ensure that you are saving the model with model.save() or
tf.keras.models.save_model(), *NOT* tf.saved_model.save(). To confirm, there should be a file
named "keras_metadata.pb" in the SavedModel directory.

WARNING:tensorflow:No training configuration found in save file, so the model was *not*
compiled. Compile it manually.

9 cells are segmented.

ans =

imgCardWholeCell with properties:

cellArray: [1×9 wholeCellCard]

>> imgCardWholeCell('DeepCell','0_00 copy.tif')

A local file was found, but it seems to be incomplete or outdated because the auto file hash
does not match the original value of 6a244f561b4d37169cb1a58b6029910f so we will
re-download the data.

Downloading data from

<https://deepcell-data.s3-us-west-1.amazonaws.com/saved-models/CytoplasmSegmentation-3.tar.gz>

....

95264768/95263450 [=====] - 29s 0us/step

WARNING:tensorflow:SavedModel saved prior to TF 2.5 detected when loading Keras model.
Please ensure that you are saving the model with model.save() or
tf.keras.models.save_model(), *NOT* tf.saved_model.save(). To confirm, there should be a file
named "keras_metadata.pb" in the SavedModel directory.

WARNING:tensorflow:No training configuration found in save file, so the model was *not*
compiled. Compile it manually.

9 cells are segmented.