LC3B_Tandem_Puncta_QuantificationV2

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Code requires bioformats MATLAB Toolbox and MATLAB version R2021a or later.

```
Creator
Created by: Joaquin Quintana (last modified: 05-18-2021)
Email: Joaquin.Quintana@Colorado.edu
```

LC3B_Tandem_Puncta_QuantificationV2 is a batch processing function used to identify LC3B vacuoles labeled with eGFP and mCherry signals and is designed for researchers investigating autophagy. Code expects the users data to be grayscale 16-bit volume data either in the form of multidimensional tiff stacks or ND2 files with three channels corresponding to the eGFP, mCherry and nuclear marker for mammalian cells. Data used to test this function was acquired using a confocal microscope as out-of-focus light will introduce issues with segmentation and other modes of image acquisition that do not minimize out-of-focus light will not produce quality results.

The file calls the functions:

userInputs imreadVolume Watershed2 FindVaculoes TabularCountVacuoles

Data for single cell analysis is reported as the number of eGFP and mCherry vacuoles counted for each cell and is printed to the command window along with the fileName the data was extracted from and the cellindex corresponding to which cell the vacuoles were found in with respect to the image used. This data is returned as a table to the command window and can also be viewed by calling or opening the structure Results_Table.

NOTE: Each cell line is different and each optical system is as well. Users should experimentally determine the parameters to use for their data. A helper function is provided with the code to help users access and identify parameters for their data called VolumeThresholdhelper.

NOTE: Users can use MATLABs publishing option to capture all the outputs (recommended) for all figures created by the subfunctions above or place a waitfor() call after each figure to observe the results of each figure created as the processing is occuring. See MATLABs documentation for publishing options and using watifor(). See github account for an example of the published output.

```
%clear the command window, users workspace and close all figures
clc;
clear;
close all;
```

Ask for userInputs

```
[Input] = userInput();
```

Create image data store

```
imds = imageDatastore(Input.Indir,...
'IncludeSubfolders',true,'FileExtensions',
{'.tif','.ND2'},'LabelSource',...
    'foldernames');
imds_NumerOfFiles = size(imds.Files(),1);
```

Initialize data storage

```
dat = struct ;
dat.FileName = [];
dat.SingleCell = [];
```

Start processing images

```
for ii = 1:imds_NumerOfFiles

%pull images from data store one at a time by indexing
img = bfopen(imds.Files{ii,:});

%get file name
[pathstr,name] = fileparts(imds.Files{ii,:});



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%let the user know which image is being processed. Print the name to
 command
%window
disp("Begin processing image: ")
disp(name)
```

```
%unpack user inputs for passing to functions
Nuclear_channel = Input.Nuclear_channel;
eGFP_channel = Input.eGFP_channel;
mCherry_channel = Input.mCherry_channel;
eGFP_threshold = Input.threshG;
mCherry_threshold = Input.threshR;
MinvacVoxeGFP = Input.minGFPvacVolume;
MinvacVoxmCherry = Input.minRFPvacVolume;
MinNucVox = Input.minNucleusVolume;
Solidity = Input.Solidity;
```

Begin processing image:
High numbers of eGFP and mCherry

Begin processing image:
Low numbers of eGFP puncta and high mCherry Puncta (2)

Begin processing image:
Low numbers of eGFP puncta and high mCherry Puncta

Begin processing image:
Low numbers of eGFP puncta and low numbers of mCherry Puncta (Noisy Test)

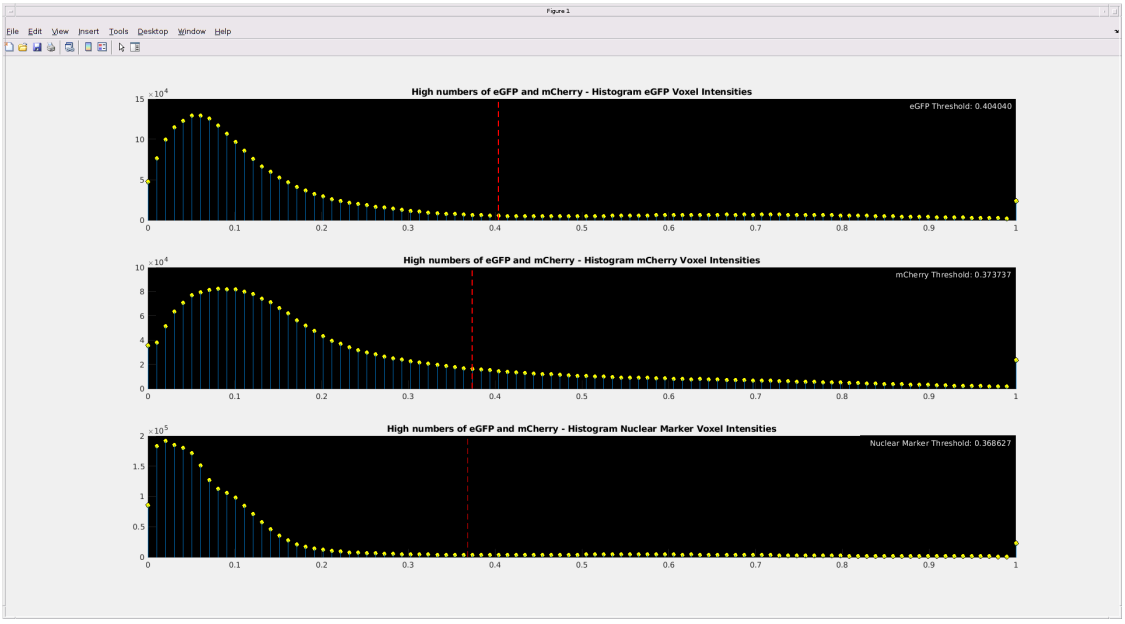
Create subvolumes for each channel

```
[r,g,b] =
 imreadVolume(img,Nuclear_channel,eGFP_channel,mCherry_channel);
```

3D controlled watershed

```
[bw_stats] = Watershed2(r,g,b,name,MinNucVox,true);
```

Data for plots generated from - High numbers of eGFP and mCherry



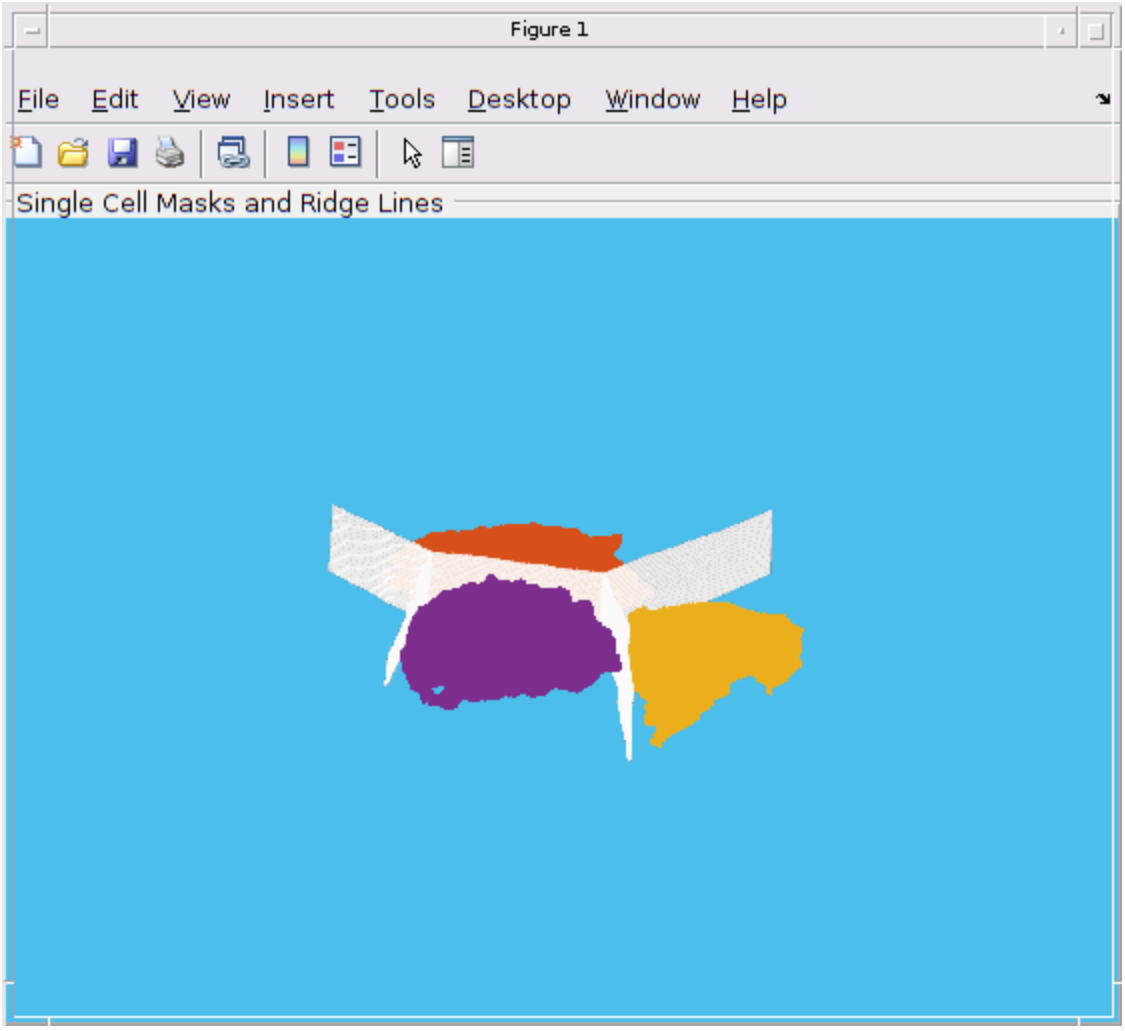
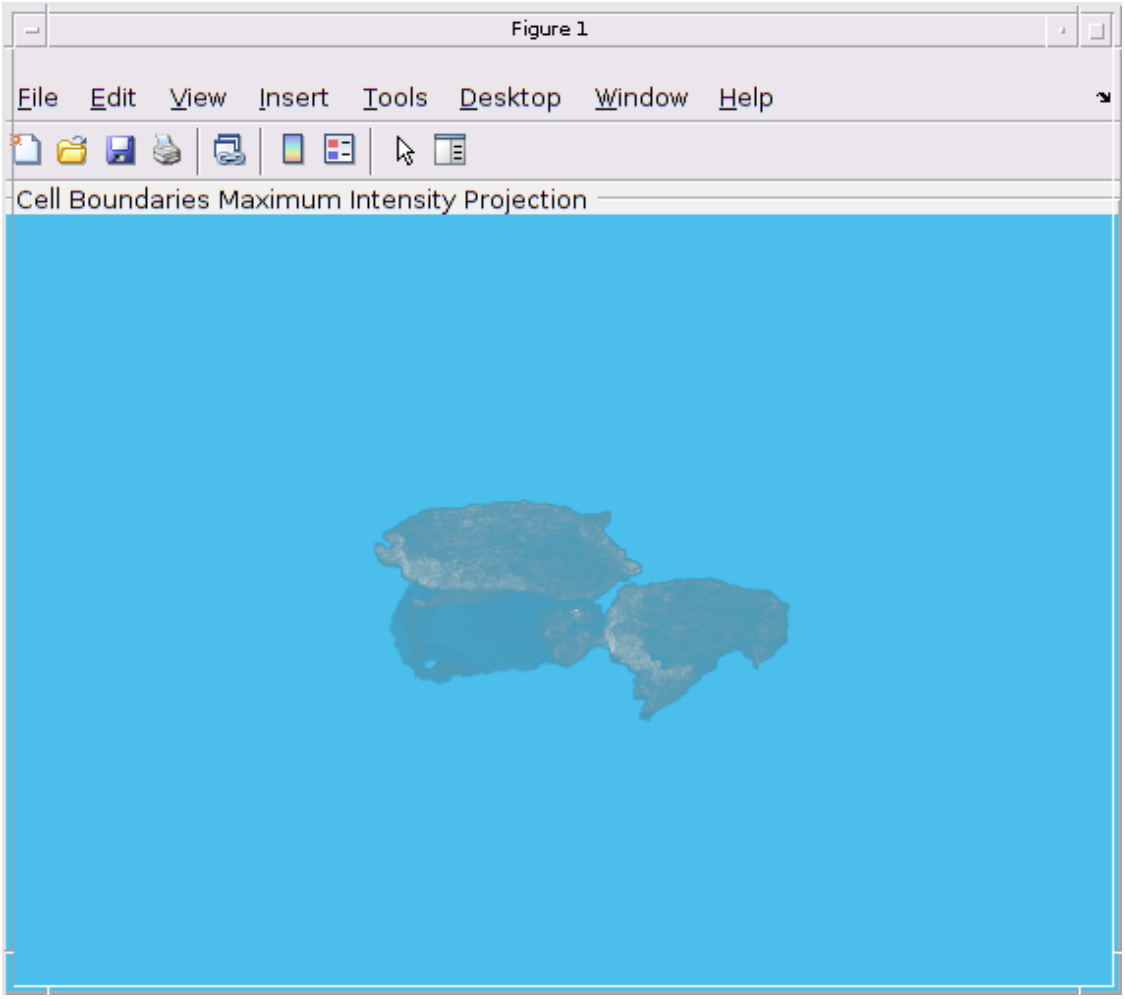


Figure 1
File Edit View Insert Tools Desktop Window Help
Single Cell Masks and Ridge Lines



Data for plots generated from - Low numbers of eGFP puncta and high mCherry Puncta (2)

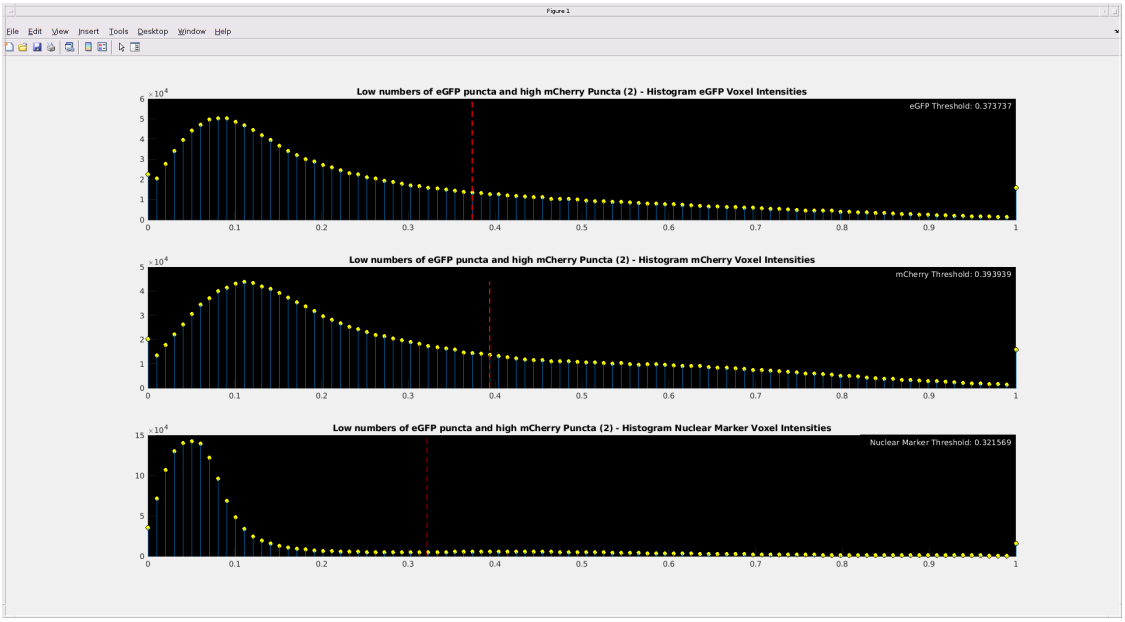


Figure 1
File Edit View Insert Tools Desktop Window Help
Low numbers of eGFP puncta and high mCherry Puncta (2) - Histogram eGFP Voxel Intensities
eGFP Threshold: 0.373737
Low numbers of eGFP puncta and high mCherry Puncta (2) - Histogram mCherry Voxel Intensities
mCherry Threshold: 0.393939
Low numbers of eGFP puncta and high mCherry Puncta (2) - Histogram Nuclear Marker Voxel Intensities
Nuclear Marker Threshold: 0.321569

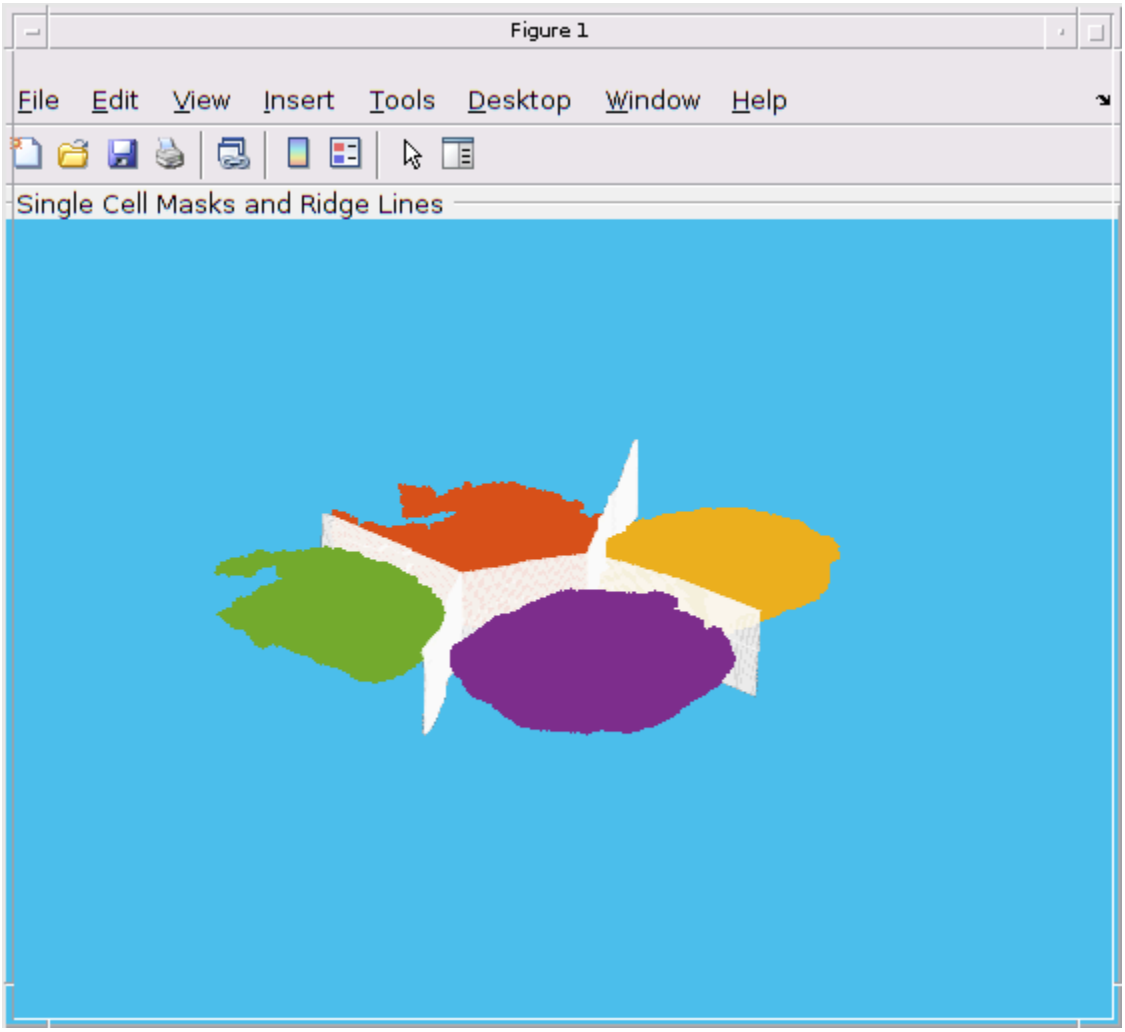
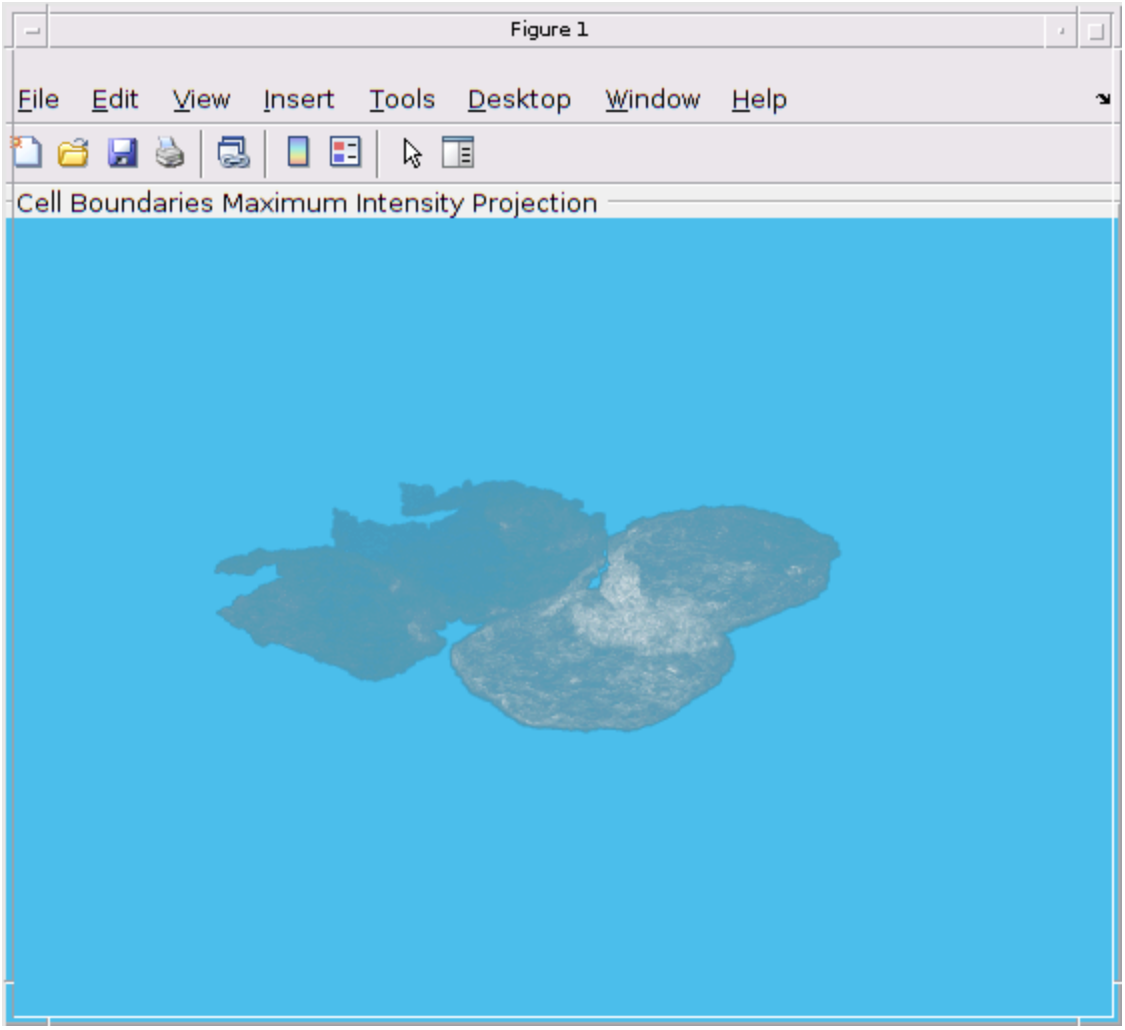


Figure 1
File Edit View Insert Tools Desktop Window Help
Single Cell Masks and Ridge Lines



Data for plots generated from - Low numbers of eGFP puncta and high mCherry Puncta

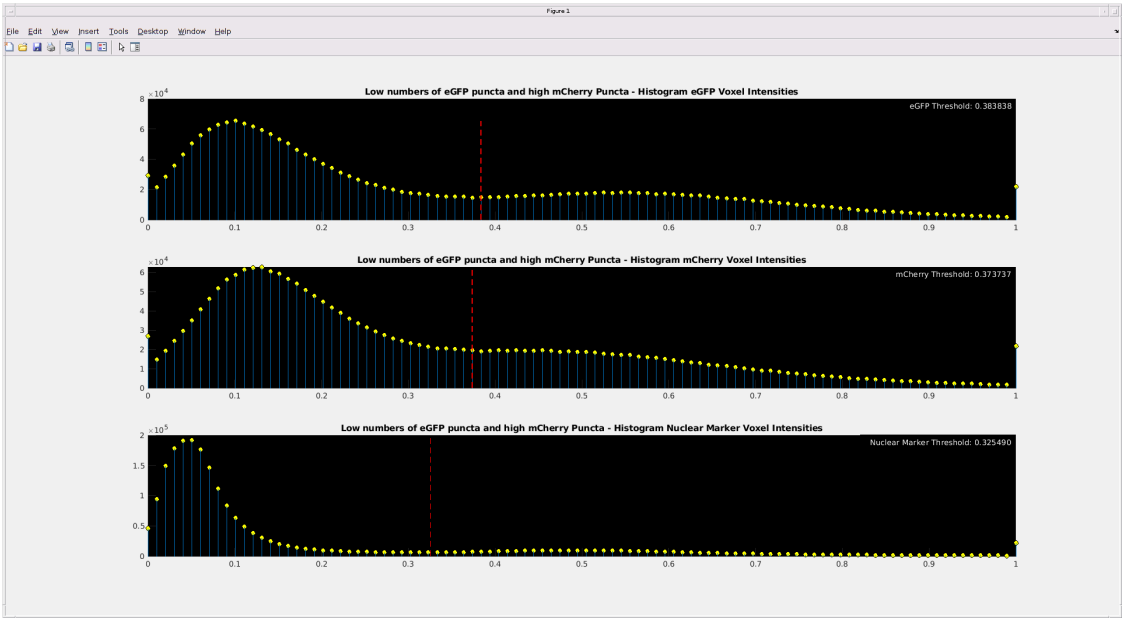


Figure 1
File Edit View Insert Tools Desktop Window Help
Low numbers of eGFP puncta and high mCherry Puncta - Histogram eGFP Voxel Intensities
eGFP Threshold: 0.383838
Low numbers of eGFP puncta and high mCherry Puncta - Histogram mCherry Voxel Intensities
mCherry Threshold: 0.373737
Low numbers of eGFP puncta and high mCherry Puncta - Histogram Nuclear Marker Voxel Intensities
Nuclear Marker Threshold: 0.325490

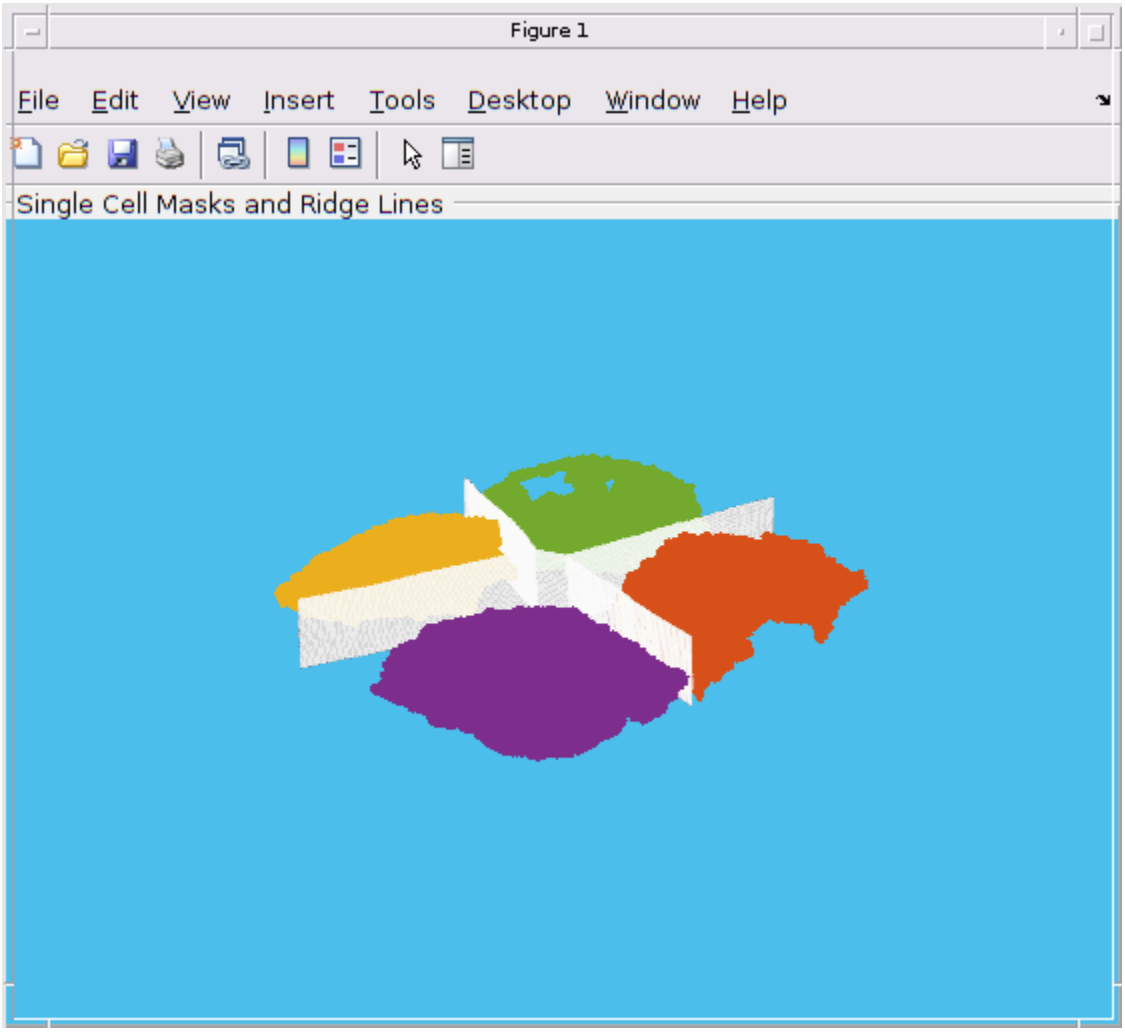
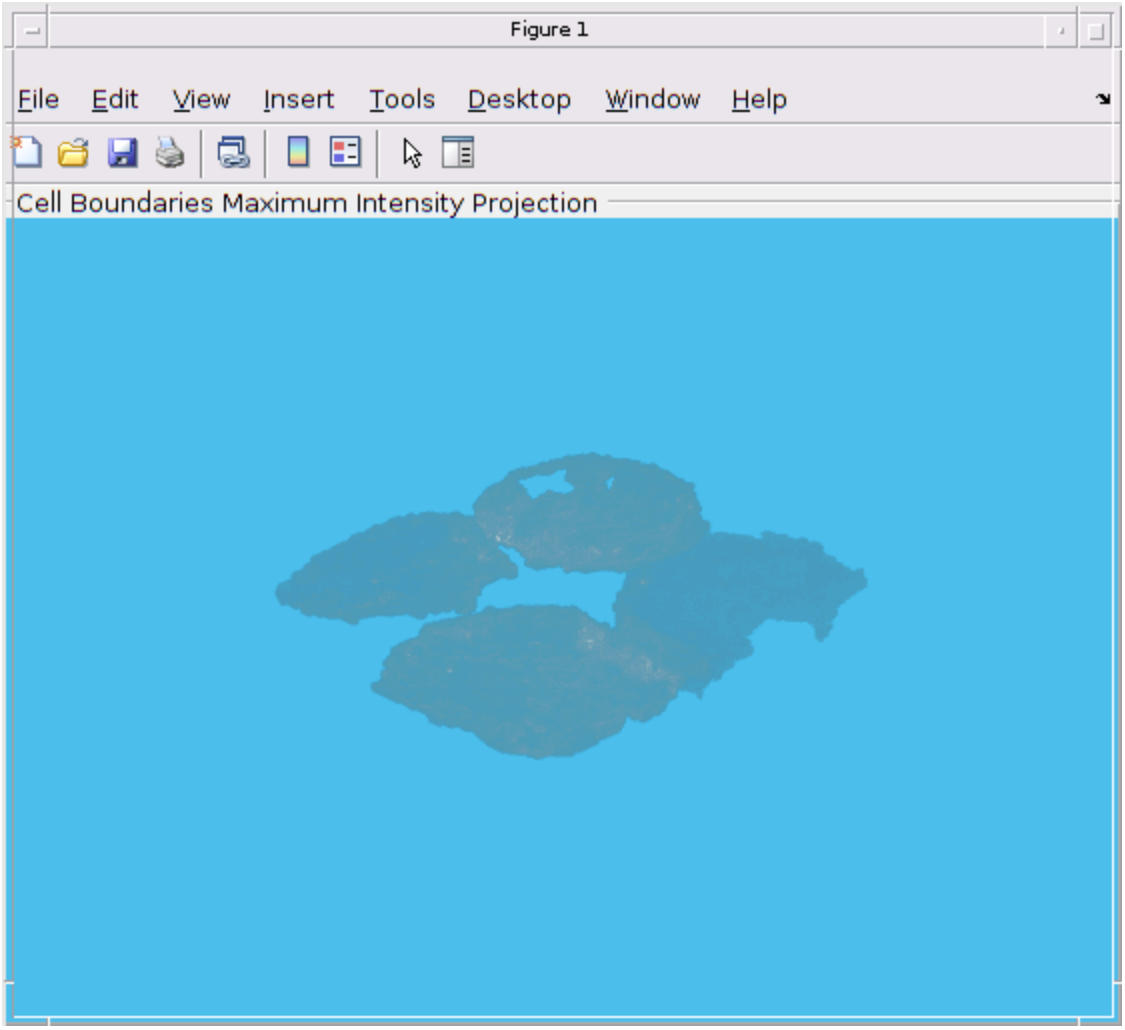


Figure 1
File Edit View Insert Tools Desktop Window Help
Single Cell Masks and Ridge Lines



Data for plots generated from - Low numbers of eGFP puncta and low numbers of mCherry Puncta (Noisy Test)

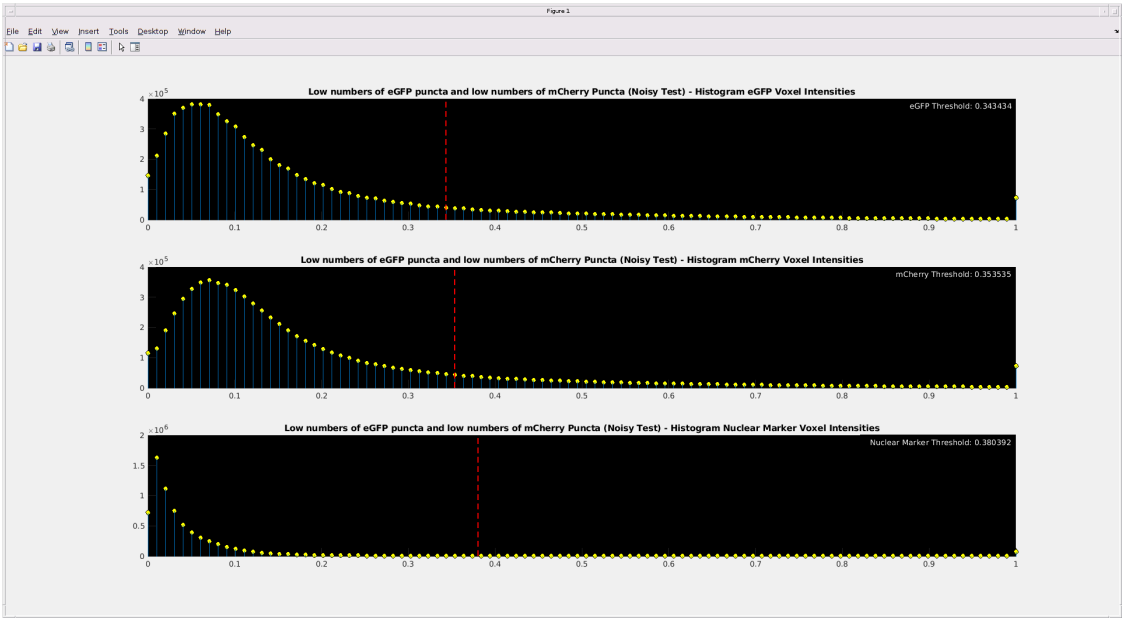


Figure 1
File Edit View Insert Tools Desktop Window Help
Low numbers of eGFP puncta and low numbers of mCherry Puncta (Noisy Test) - Histogram eGFP Voxel Intensities
eGFP Threshold: 0.343434
Low numbers of eGFP puncta and low numbers of mCherry Puncta (Noisy Test) - Histogram mCherry Voxel Intensities
mCherry Threshold: 0.353535
Low numbers of eGFP puncta and low numbers of mCherry Puncta (Noisy Test) - Histogram Nuclear Marker Voxel Intensities
Nuclear Marker Threshold: 0.380392

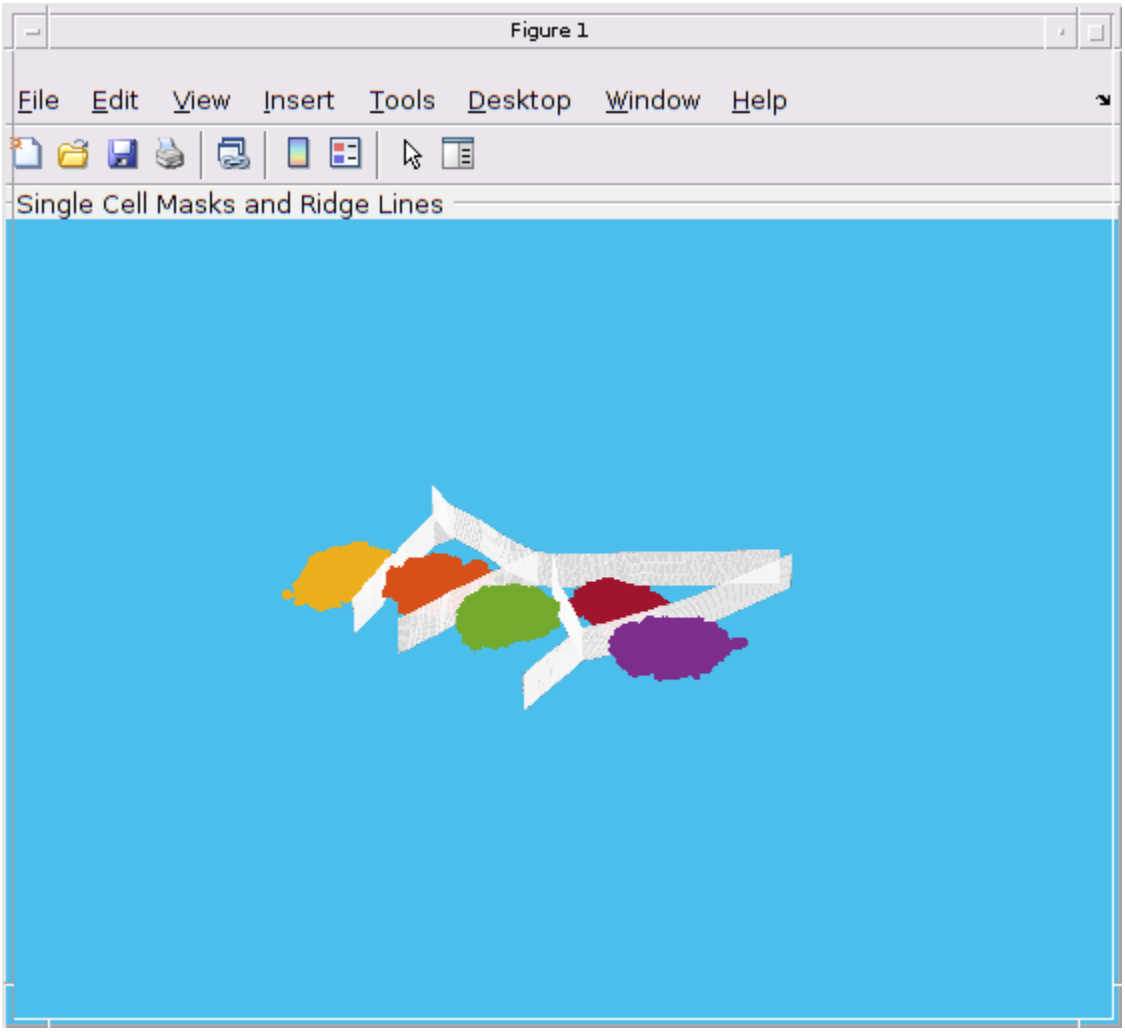
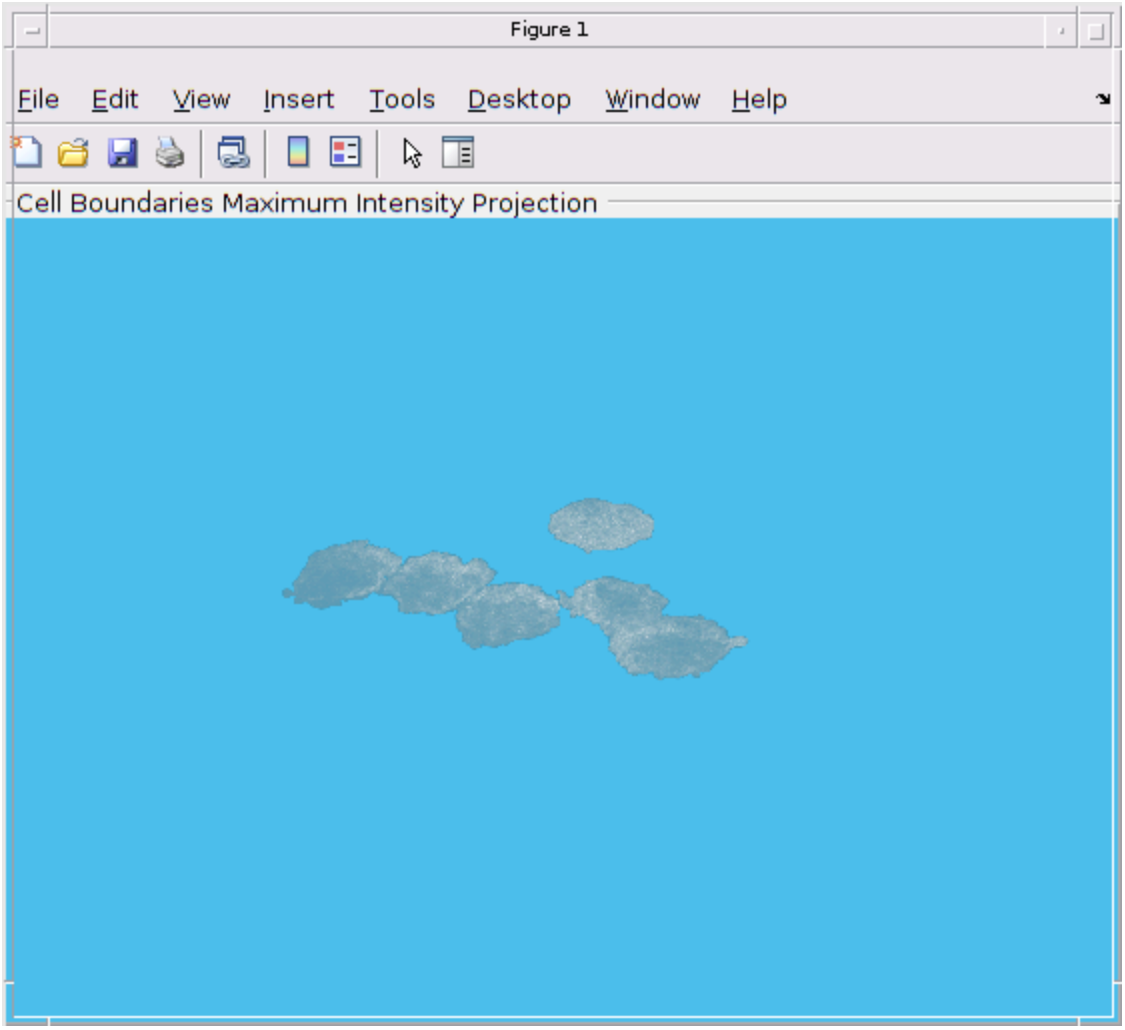


Figure 1
File Edit View Insert Tools Desktop Window Help
Single Cell Masks and Ridge Lines



Find the vacuoles for each cell identified

```
[SingleCellStructure] =
 FindVaculoes(r,g,b,bw_stats,name,eGFP_threshold,...

 mCherry_threshold,MinvacVoxeGFP,MinvacVoxmCherry,MinNucVox,Solidity);
```

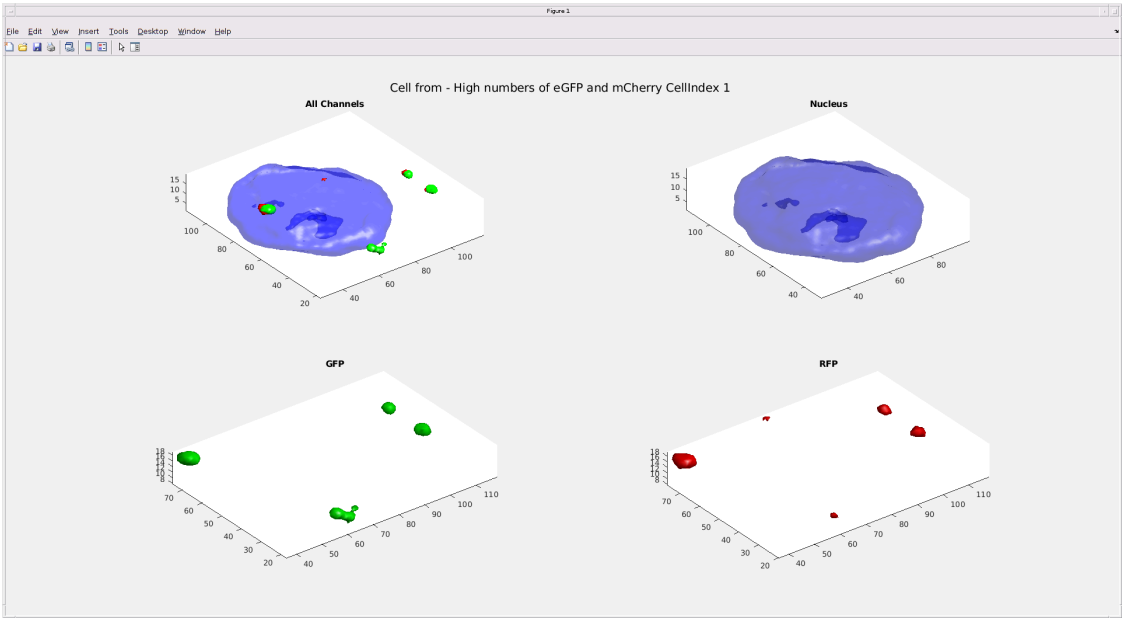


Figure 1
File Edit View Insert Tools Desktop Window Help
Cell from - High numbers of eGFP and mCherry CellIndex 1
All Channels
Nucleus
GFP
RFP

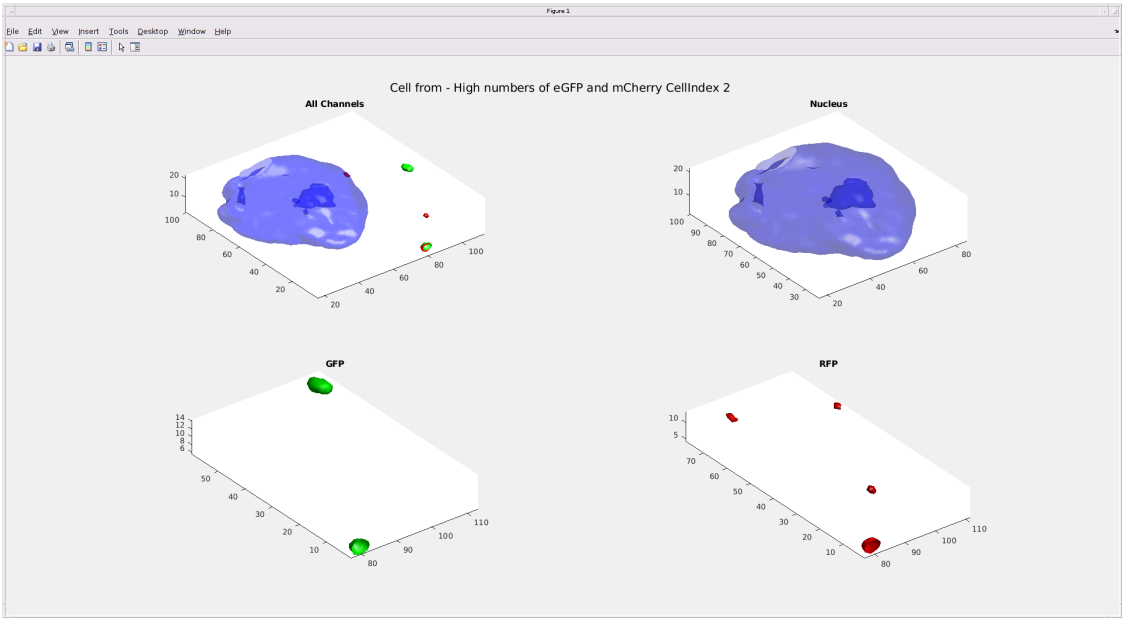


Figure 1
File Edit View Insert Tools Desktop Window Help
Cell from - High numbers of eGFP and mCherry CellIndex 2
All Channels
Nucleus
GFP
RFP

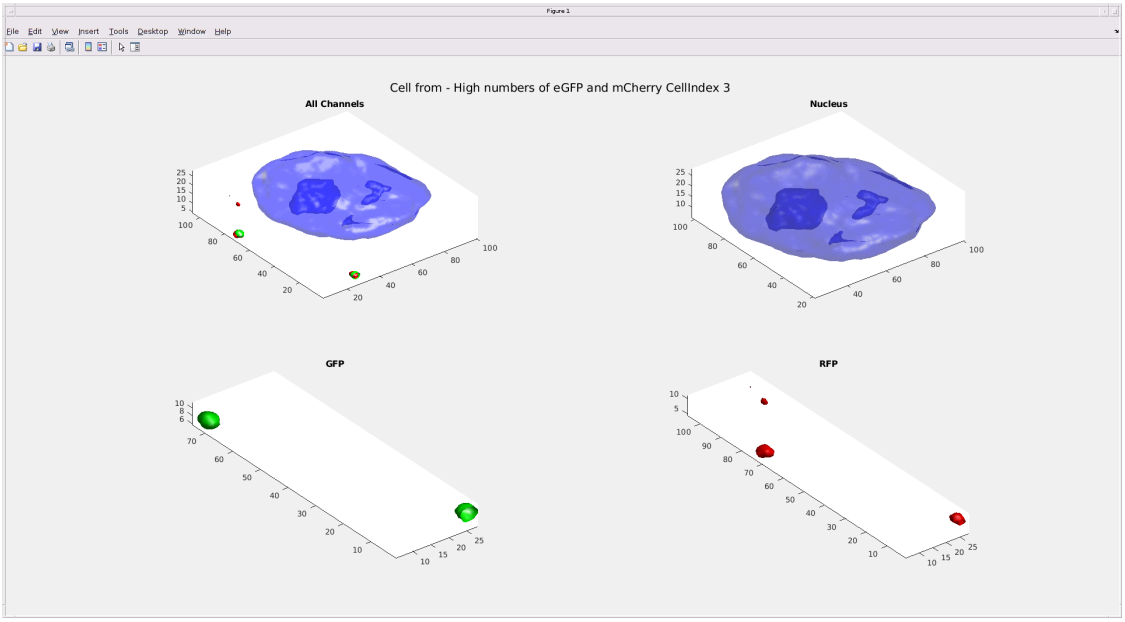


Figure 1
File Edit View Insert Tools Desktop Window Help
Cell from - High numbers of eGFP and mCherry CellIndex 3
All Channels
Nucleus
GFP
RFP

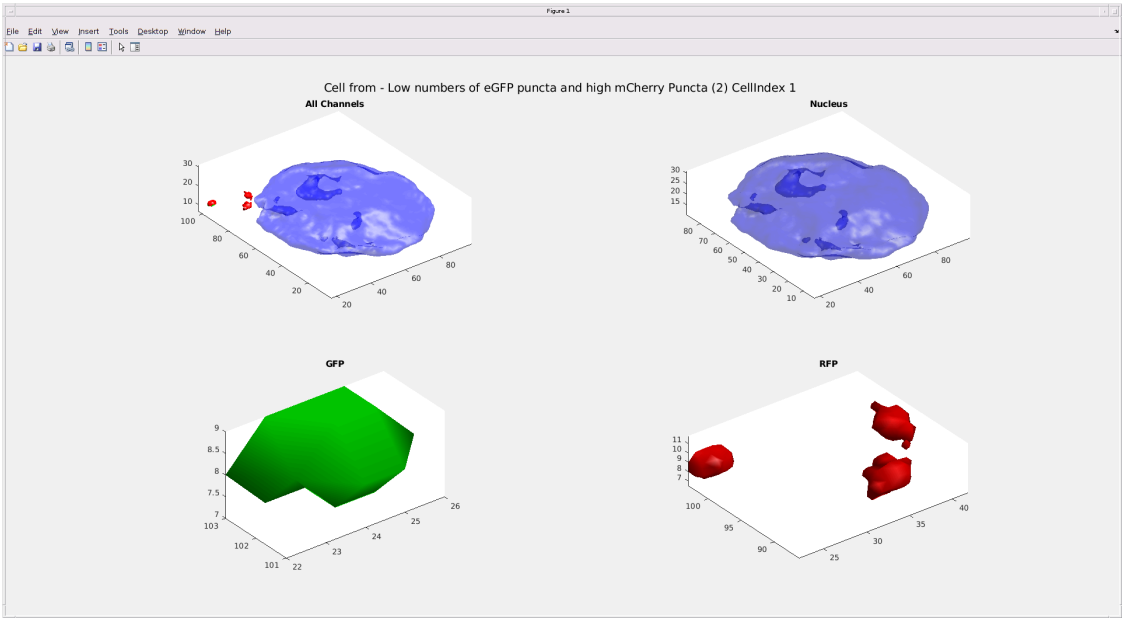


Figure 1
File Edit View Insert Tools Desktop Window Help
Cell from - Low numbers of eGFP puncta and high mCherry Puncta (2) CellIndex 1
All Channels
Nucleus
GFP
RFP

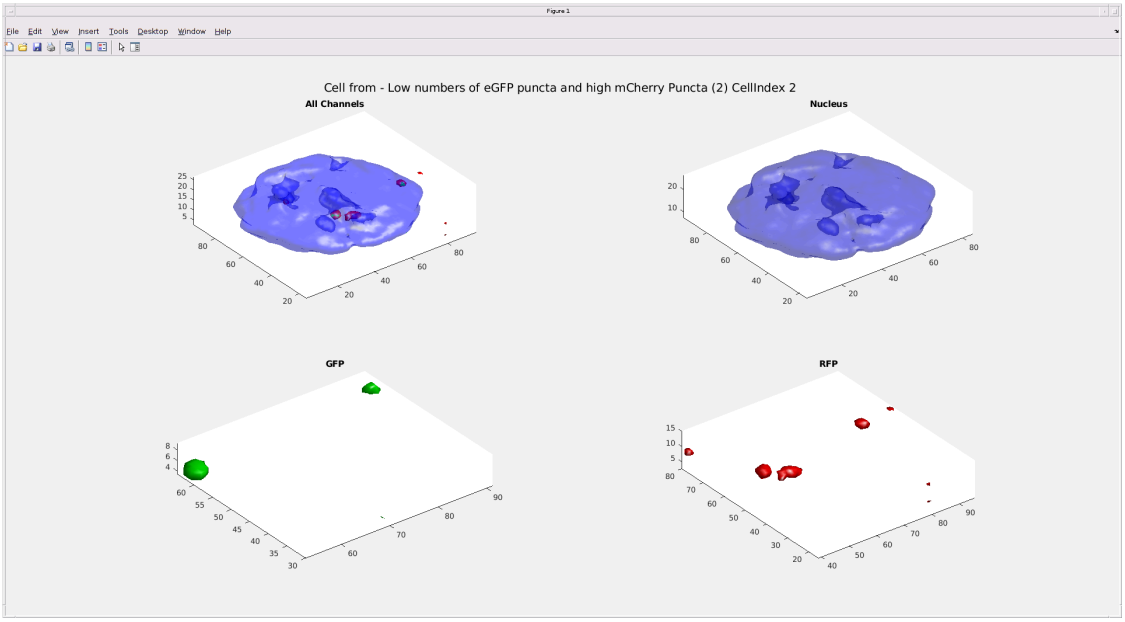


Figure 1
File Edit View Insert Tools Desktop Window Help
Cell from - Low numbers of eGFP puncta and high mCherry Puncta (2) CellIndex 2
All Channels
Nucleus
GFP
RFP

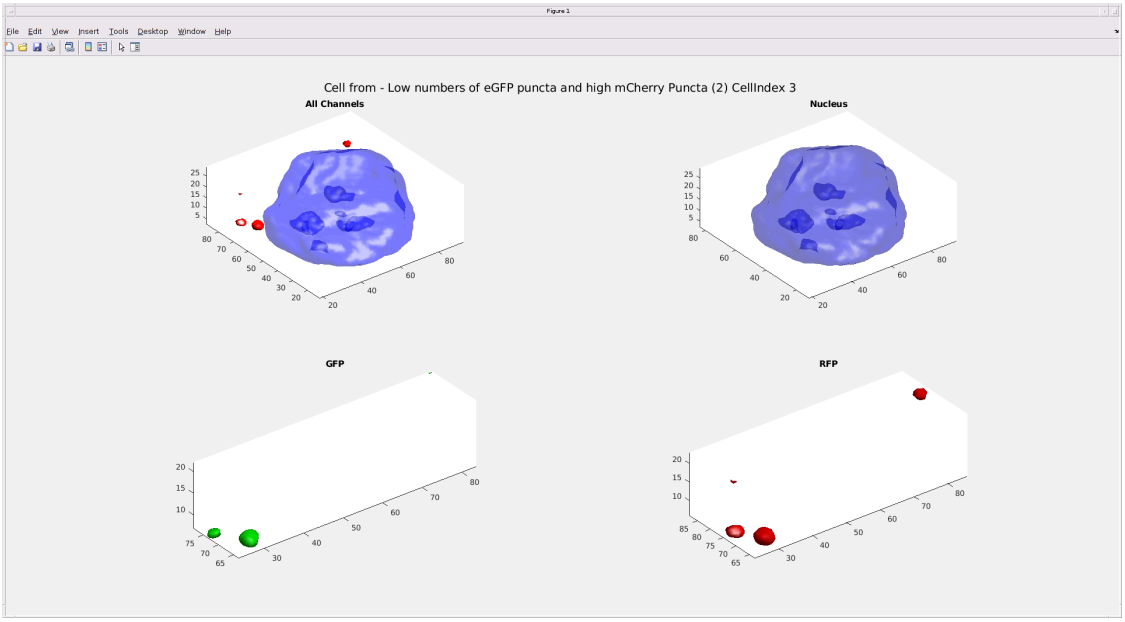


Figure 1
File Edit View Insert Tools Desktop Window Help
Cell from - Low numbers of eGFP puncta and high mCherry Puncta (2) CellIndex 3
All Channels
Nucleus
GFP
RFP

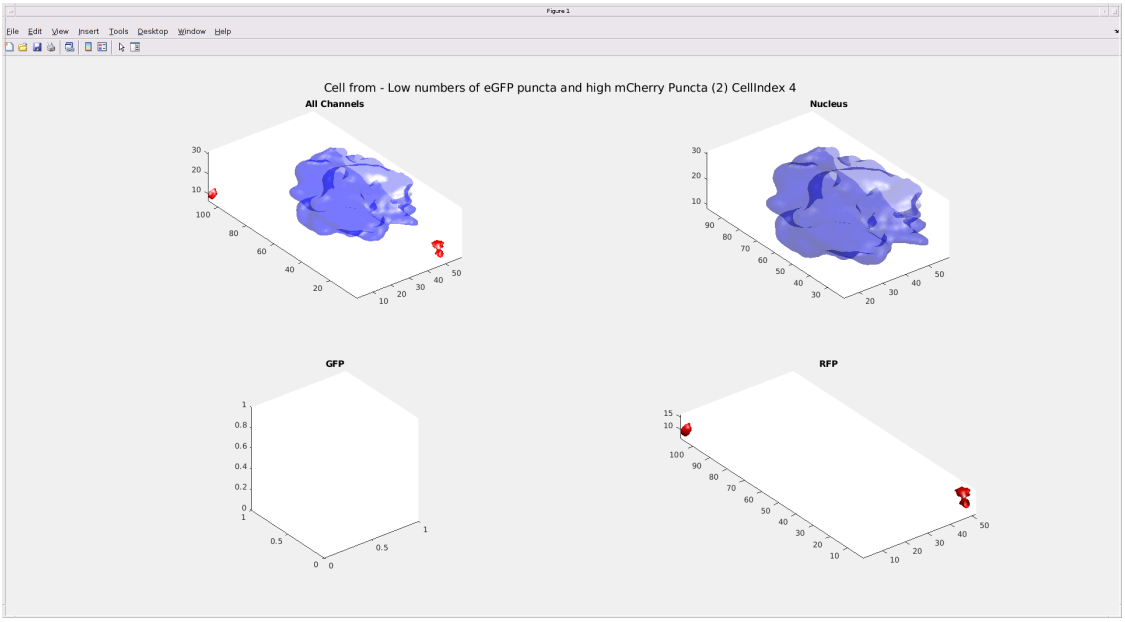


Figure 1
File Edit View Insert Tools Desktop Window Help
Cell from - Low numbers of eGFP puncta and high mCherry Puncta (2) CellIndex 4
All Channels
Nucleus
GFP
RFP

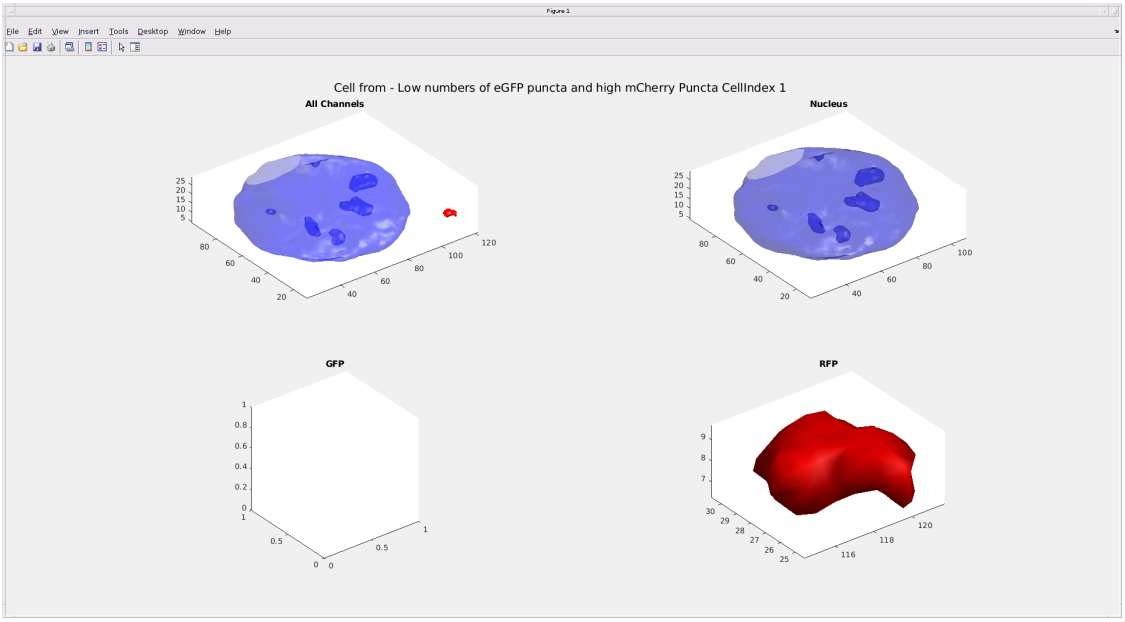


Figure 1
File Edit View Insert Tools Desktop Window Help
Cell from - Low numbers of eGFP puncta and high mCherry Puncta CellIndex 1
All Channels
Nucleus
GFP
RFP

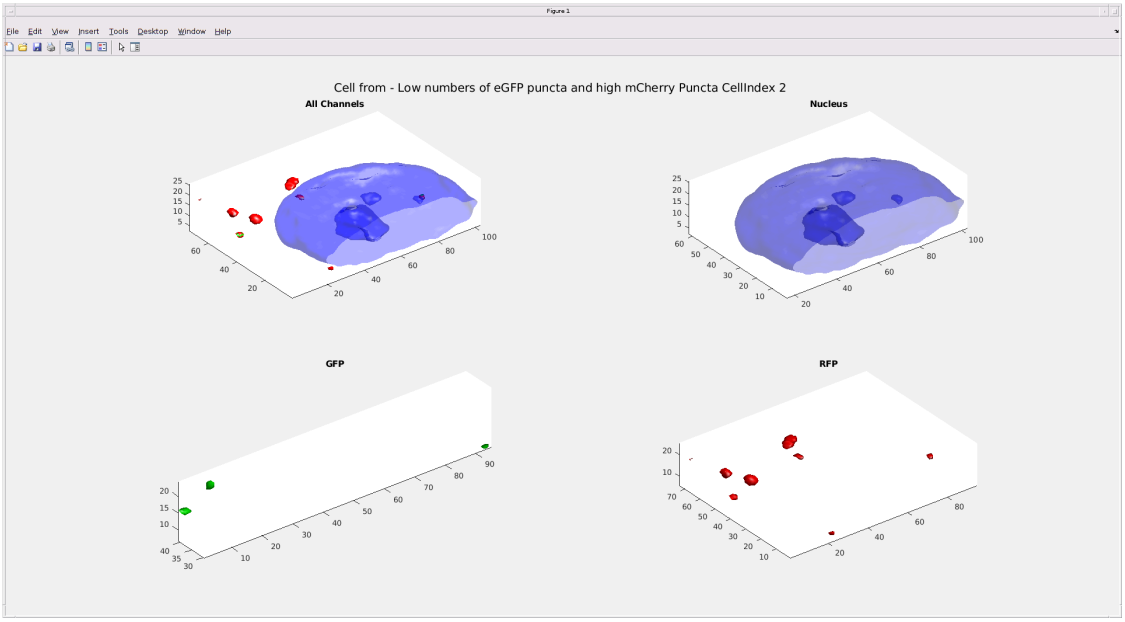


Figure 1
File Edit View Insert Tools Desktop Window Help
Cell from - Low numbers of eGFP puncta and high mCherry Puncta CellIndex 2
All Channels
Nucleus
GFP
RFP

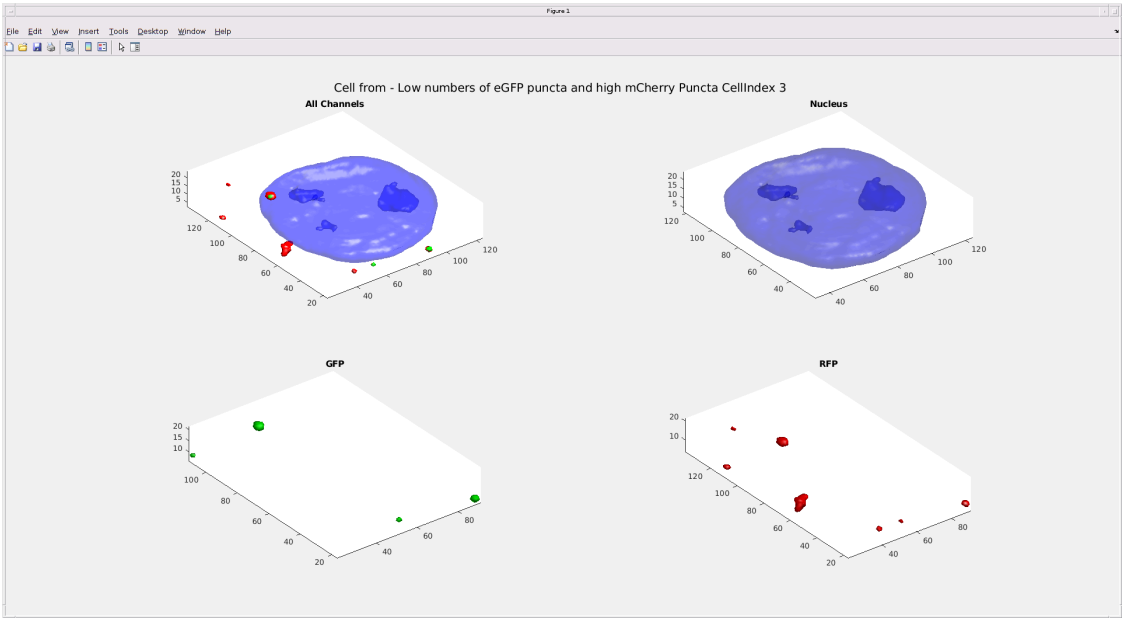


Figure 1
File Edit View Insert Tools Desktop Window Help
Cell from - Low numbers of eGFP puncta and high mCherry Puncta CellIndex 3
All Channels
Nucleus
GFP
RFP

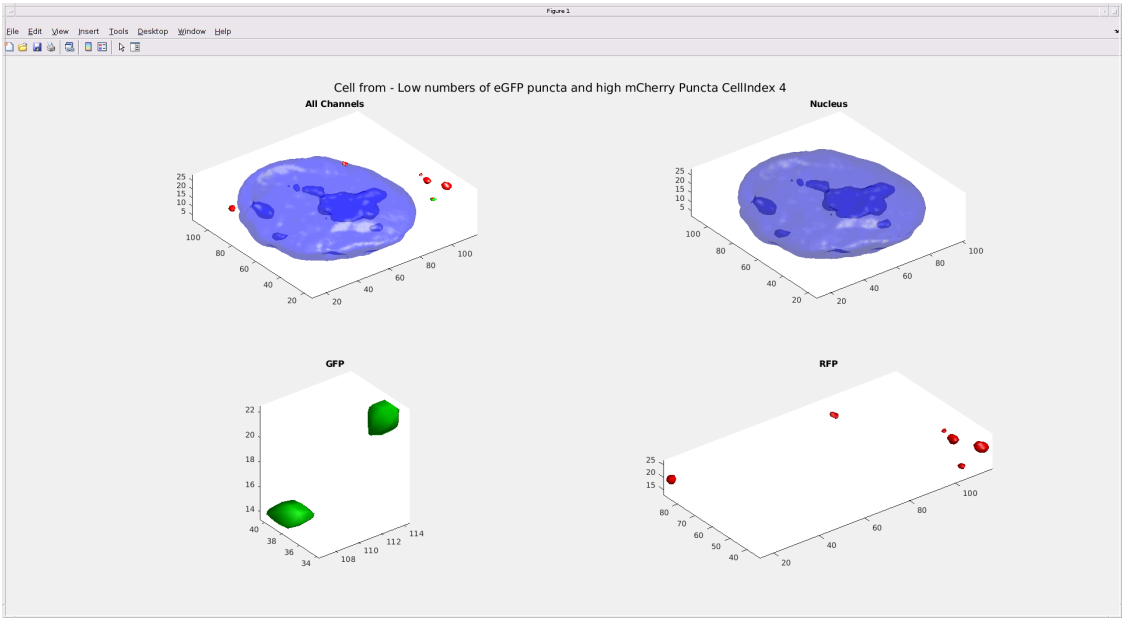


Figure 1
File Edit View Insert Tools Desktop Window Help
Cell from - Low numbers of eGFP puncta and high mCherry Puncta CellIndex 4
All Channels
Nucleus
GFP
RFP

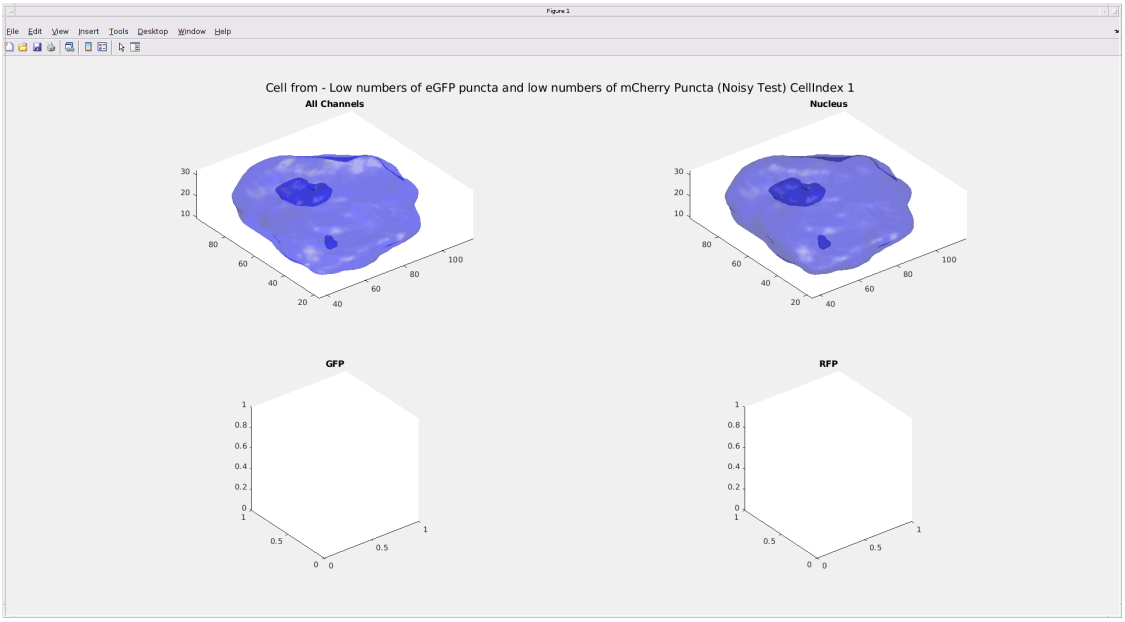


Figure 1
File Edit View Insert Tools Desktop Window Help
Cell from - Low numbers of eGFP puncta and low numbers of mCherry Puncta (Noisy Test) CellIndex 1
All Channels
Nucleus
GFP
RFP

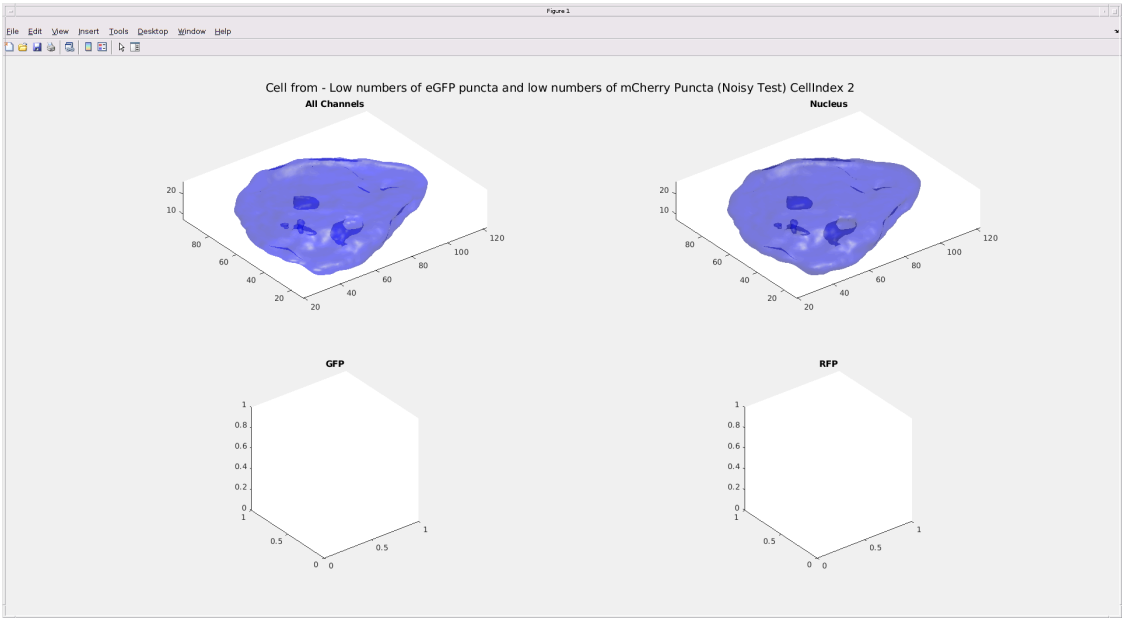


Figure 1
File Edit View Insert Tools Desktop Window Help
Cell from - Low numbers of eGFP puncta and low numbers of mCherry Puncta (Noisy Test) CellIndex 2
All Channels
Nucleus
GFP
RFP

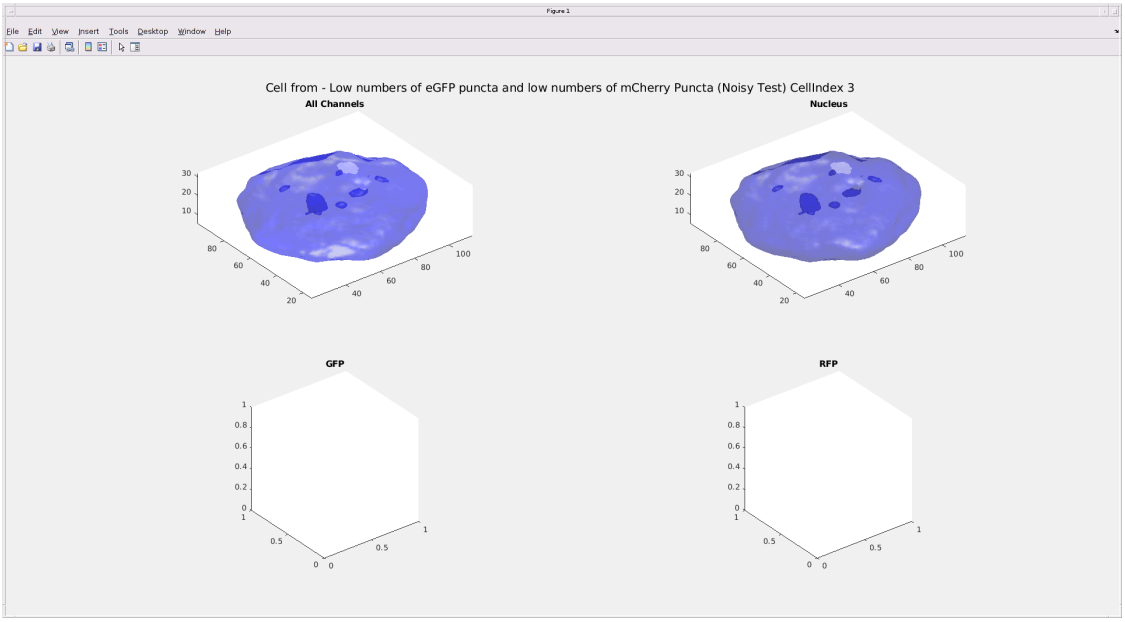


Figure 1
File Edit View Insert Tools Desktop Window Help
Cell from - Low numbers of eGFP puncta and low numbers of mCherry Puncta (Noisy Test) CellIndex 3
All Channels
Nucleus
GFP
RFP

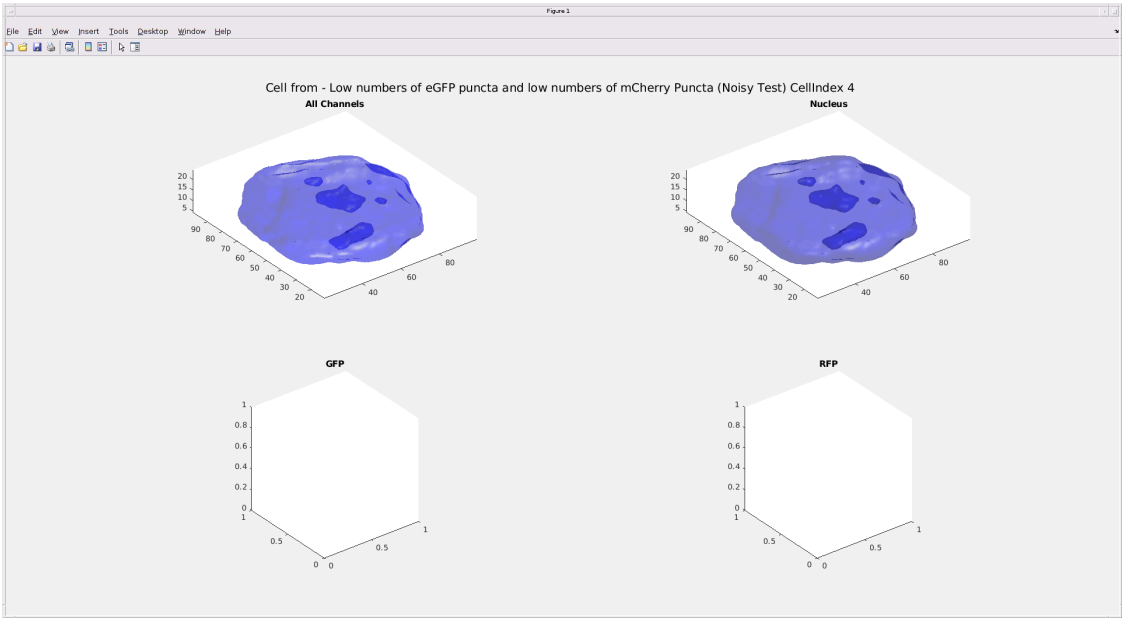


Figure 1
File Edit View Insert Tools Desktop Window Help
Cell from - Low numbers of eGFP puncta and low numbers of mCherry Puncta (Noisy Test) CellIndex 4
All Channels
Nucleus
GFP
RFP

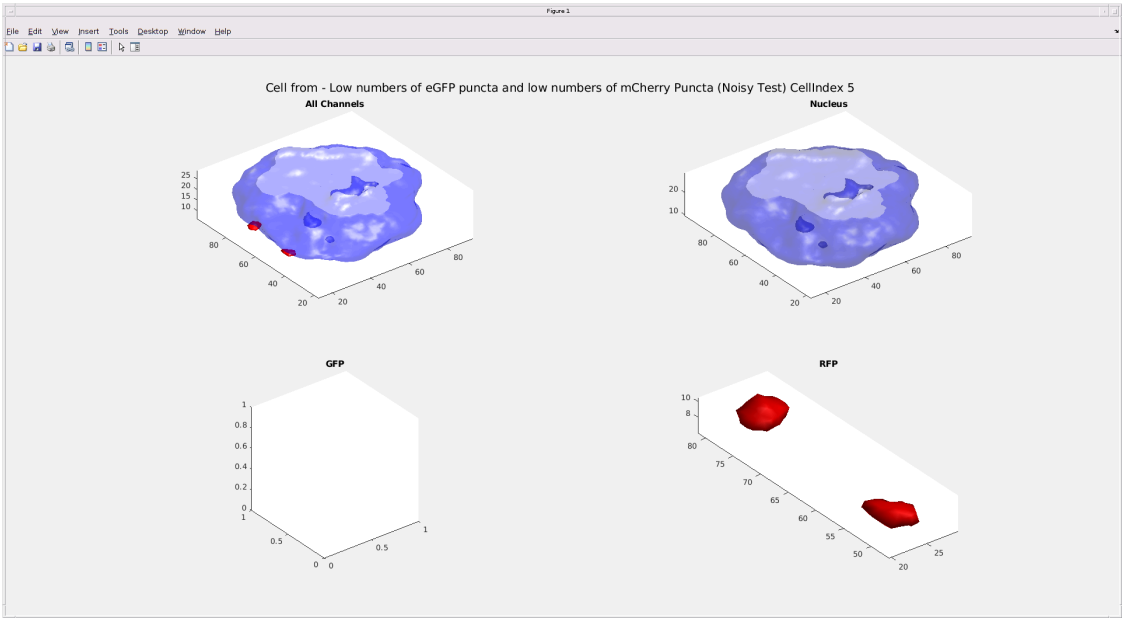
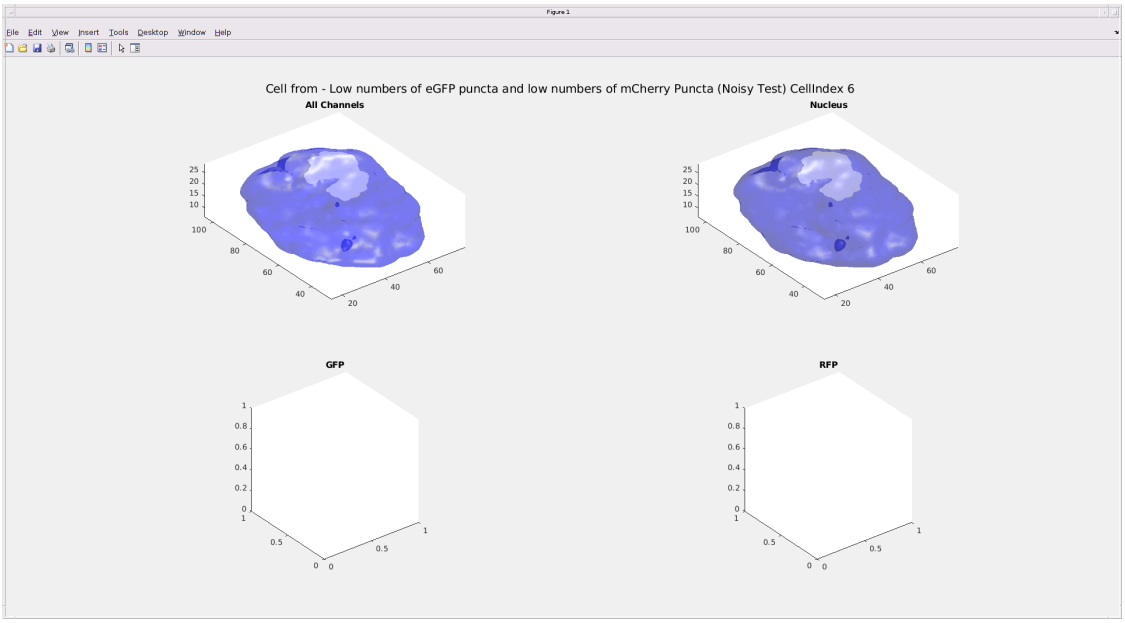


Figure 1
File Edit View Insert Tools Desktop Window Help
Cell from - Low numbers of eGFP puncta and low numbers of mCherry Puncta (Noisy Test) CellIndex 5
All Channels
Nucleus
GFP
RFP



Accumulate results

```
dat(ii).FileName = name;
dat(ii).SingleCell = SingleCellStructure;

end
```

Return counts for vacuoles as table

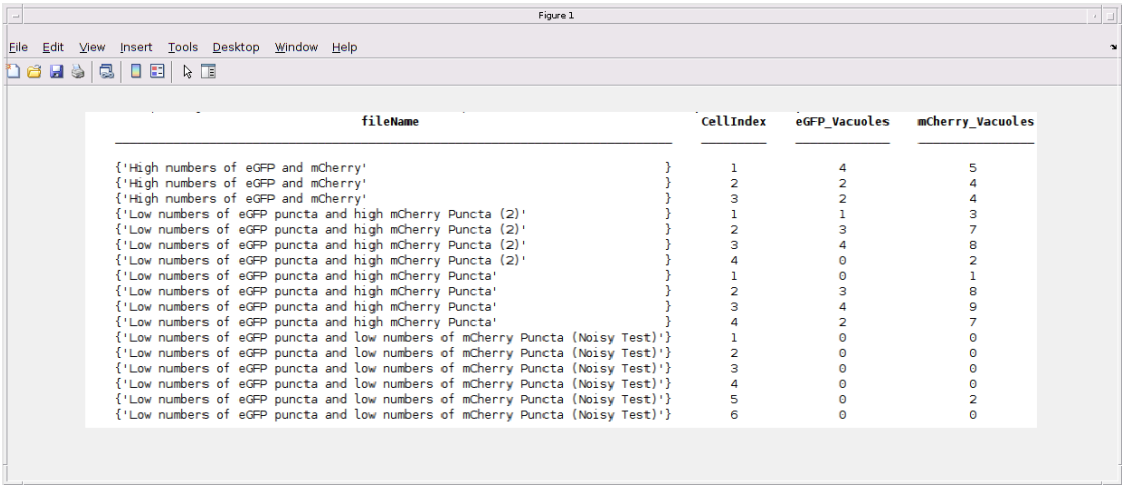
```
close all;
[Results_Table] = TabularCountVacuoles(dat);

disp(Results_Table)
imshow('Results.png')
snapnow
```

```
                                                fileName
                    CellIndex    eGFP_Vacuoles    mCherry_Vacuoles
    ____________    _________    _____________    ________________

    {'High numbers of eGFP and mCherry'
          }         1                4                5
    {'High numbers of eGFP and mCherry'
          }         2                2                4
    {'High numbers of eGFP and mCherry'
          }         3                2                4
    {'Low numbers of eGFP puncta and high mCherry Puncta (2)'
          }         1                1                3
    {'Low numbers of eGFP puncta and high mCherry Puncta (2)'
          }         2                3                7
```

```
    {'Low numbers of eGFP puncta and high mCherry Puncta (2)'
          }        3                4                   8
    {'Low numbers of eGFP puncta and high mCherry Puncta (2)'
          }        4                0                   2
    {'Low numbers of eGFP puncta and high mCherry Puncta'
          }        1                0                   1
    {'Low numbers of eGFP puncta and high mCherry Puncta'
          }        2                3                   8
    {'Low numbers of eGFP puncta and high mCherry Puncta'
          }        3                4                   9
    {'Low numbers of eGFP puncta and high mCherry Puncta'
          }        4                2                   7
    {'Low numbers of eGFP puncta and low numbers of mCherry Puncta
(Noisy Test)'}         1                0                   0
    {'Low numbers of eGFP puncta and low numbers of mCherry Puncta
(Noisy Test)'}         2                0                   0
    {'Low numbers of eGFP puncta and low numbers of mCherry Puncta
(Noisy Test)'}         3                0                   0
    {'Low numbers of eGFP puncta and low numbers of mCherry Puncta
(Noisy Test)'}         4                0                   0
    {'Low numbers of eGFP puncta and low numbers of mCherry Puncta
(Noisy Test)'}         5                0                   2
    {'Low numbers of eGFP puncta and low numbers of mCherry Puncta
(Noisy Test)'}         6                0                   0
```



fileName	CellIndex	eGFP_Vacuoles	mCherry_Vacuoles
{'High numbers of eGFP and mCherry'}	1	4	5
{'High numbers of eGFP and mCherry'}	2	2	4
{'High numbers of eGFP and mCherry'}	3	2	4
{'Low numbers of eGFP puncta and high mCherry Puncta (2)'}	1	1	3
{'Low numbers of eGFP puncta and high mCherry Puncta (2)'}	2	3	7
{'Low numbers of eGFP puncta and high mCherry Puncta (2)'}	3	4	8
{'Low numbers of eGFP puncta and high mCherry Puncta (2)'}	4	0	2
{'Low numbers of eGFP puncta and high mCherry Puncta'}	1	0	1
{'Low numbers of eGFP puncta and high mCherry Puncta'}	2	3	8
{'Low numbers of eGFP puncta and high mCherry Puncta'}	3	4	9
{'Low numbers of eGFP puncta and high mCherry Puncta'}	4	2	7
{'Low numbers of eGFP puncta and low numbers of mCherry Puncta (Noisy Test)'}	1	0	0
{'Low numbers of eGFP puncta and low numbers of mCherry Puncta (Noisy Test)'}	2	0	0
{'Low numbers of eGFP puncta and low numbers of mCherry Puncta (Noisy Test)'}	3	0	0
{'Low numbers of eGFP puncta and low numbers of mCherry Puncta (Noisy Test)'}	4	0	0
{'Low numbers of eGFP puncta and low numbers of mCherry Puncta (Noisy Test)'}	5	0	2
{'Low numbers of eGFP puncta and low numbers of mCherry Puncta (Noisy Test)'}	6	0	0

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