**RNAscope Human ANALYSIS STEPS**

1. Structure of the code

D**ata processing**

R code for running CART algorithm to categorize nuclei

**ROI detection**

Matlab code for preprocessing ROI (Nuclei) and dot (transcripts) segmentation

**Input file**

Czi file with DAPI and gene channels

**Data analysis**

R code to build graphs and perform stats

1. Description

**ROI detection:** This part of matlab code takes in the .czi file, processes and saves the output (segmented nuclei and dot metrics) as mat files where the the input ‘.czi’ is located. The output mat file is a Matlab structure with each channel as a field.

**Eg:**

**input file**: ‘/dcl01/lieber/ajaffe/Maddy/RNAscope/dotdot\_vignette/dotdot\_vignette/Human2.czi’

**Output:** 1.’/dcl01/lieber/ajaffe/Maddy/RNAscope/dotdot\_vignette/dotdot\_vignette/Human2\_img.mat’ (raw image)

2.’/dcl01/lieber/ajaffe/Maddy/RNAscope/dotdot\_vignette/dotdot\_vignette/Human2\_dots\_of\_ROI.mat’ (colocalization of different genes with nuclei)

3.’/dcl01/lieber/ajaffe/Maddy/RNAscope/dotdot\_vignette/dotdot\_vignette/Human2\_total\_dots.mat’ (total segmented dots in each channel)

4.’/dcl01/lieber/ajaffe/Maddy/RNAscope/dotdot\_vignette/Human2\_segmentation.mat’ (segmented image of each channel)

**Data processing:** The above ‘\*dots\_of\_ROI.mat**’** file is converted to csv (rows being each nuclei and columns are dot count in each channel) and the absolute proportion of each gene (ratio of number of voxels of gene in nuclei and total nuclei voxels) is given to the R CART model for cell type predictions.

**Running the code:**

Download the toolbox directory and assign the paths to input file and toolbox to Matlab variables as shown below. The toolbox should be added to the Matlab’s current working directory to run the code.

**filename = '/dcl01/lieber/ajaffe/Maddy/RNAscope/dotdot\_vignette/dotdot\_vignette/Human2.czi';**

**toolbox = '/dcl01/lieber/ajaffe/Maddy/RNAscope/dotdot\_vignette/dotdot\_vignette/toolbox';**

**addpath(genpath(toolbox))** %adding toolbox path to current working directory

The command ‘rnascope\_human’ takes in the filename and toolbox as the input and outputs the above mentioned mat files.

**rnascope\_human(filename, toolbox)**

**OUTPUT**

>> filename = '/dcl01/lieber/ajaffe/Maddy/RNAscope/dotdot\_vignette/dotdot\_vignette/Human2.czi';

>> toolbox = '/dcl01/lieber/ajaffe/Maddy/RNAscope/dotdot\_vignette/dotdot\_vignette/toolbox';

>> addpath(genpath(toolbox)) %adding toolbox path to current working directory

>> rnascope\_human(filename, toolbox)

Reading Images: 1 of 70 Frames

Reading Images: 2 of 70 Frames

Reading Images: 3 of 70 Frames

Reading Images: 4 of 70 Frames

Reading Images: 5 of 70 Frames

Reading Images: 6 of 70 Frames

Reading Images: 7 of 70 Frames

Reading Images: 8 of 70 Frames

Reading Images: 9 of 70 Frames

Reading Images: 10 of 70 Frames

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Reading Images: 65 of 70 Frames

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Reading Images: 69 of 70 Frames

Reading Images: 70 of 70 Frames

extracted

'DAPILp3'

'Opal520\_Lp20'

'Opal570Lp1\_0'

'Opal620\_LP10'

'Opal690Lp30'

'No103\_Lipofuscin\_63x'

Elapsed time is 5.250257 seconds.

segmented DAPILp3: 5

segmented Opal520\_Lp20: 155

segmented Opal570Lp1\_0: 122

segmented Opal620\_LP10: 2048

segmented Opal690Lp30: 954

segmented No103\_Lipofuscin\_63x: 176

Completed Masking DAPILp3

Completed Masking Opal520\_Lp20

Completed Masking Opal570Lp1\_0

Completed Masking Opal620\_LP10

Completed Masking Opal690Lp30

Completed Masking No103\_Lipofuscin\_63x

Started Opal520\_Lp20

1 cells finished in time 0.57048s

2 cells finished in time 0.80605s

3 cells finished in time 0.89423s

4 cells finished in time 0.98938s

5 cells finished in time 1.0881s

Started Opal570Lp1\_0

1 cells finished in time 0.12922s

2 cells finished in time 0.19549s

3 cells finished in time 0.27868s

4 cells finished in time 0.33056s

5 cells finished in time 0.38089s

Started Opal620\_LP10

1 cells finished in time 0.8361s

2 cells finished in time 1.6512s

3 cells finished in time 2.4203s

4 cells finished in time 3.0615s

5 cells finished in time 3.7481s

Started Opal690Lp30

1 cells finished in time 0.37236s

2 cells finished in time 0.75768s

3 cells finished in time 1.1484s

4 cells finished in time 1.4843s

5 cells finished in time 1.8443s