This is the readme file for the code included for the project: **Neuronal programming by microbiota enables environmental regulation of intestinal physiology** by *Yuuki Obata, Alvaro Castano Medina, Stefan Boeing, Ana Bon-Frauches, Werend Boesmans, Candice Fung, Mercedes Gomez de Aguero, Bahtiyar Yilmaz, Rita Lopes, Almaz Huseynova, Todd Fallesen, Stuart Horswell, Muralidhara Rao Maradana, Pieter Vanden Berghe, Andrew Murray, Brigitta Stockinger, Andrew Macpherson, and Vassilis Pachnis*.

Testing and Timing computer is a Dell Precision 7820 Workstation, Intel Zeon Silver 4114 @2.20 GHz (40 CPUs), 132 Gb memory. The OS is Windows 10, Cell Profiler Version 3.1.8, MATLAB 2018b. GPU acceleration wasn't used.

The test image file used for this pipeline is: R333 WT+3MC 40x 001_A01_G007_0001.oir

The following code files and folders are included for use and testing:

File name	Purpose	Time to run
save_all_windows.ijm	FIJI macro for splitting and saving oir files into single channel, single plane, single timepoint tiff images	<5 seconds in FIJI
python_renamer.py	Rename files if there are more than 10 z planes, such that 0 becomes 00, 1 becomes 01, 2 becomes 02…so that cell profiler reads 07, 08, 09, 10, not 0, 10, 1, 2.	<1 second in python.
Yuuki_Alvaro_project_2_3D.cpproj	Cell profiler pipeline for analysis. See manual for details.	2 minutes in CellProfiler on test image set
Cell_Profiler_3D_result_analysis.m	MATLAB script for generating spreadsheet from cell profiler data. See manual for details.	29.89s
Cell_Profiler_3D_result_analysis_mac.m	Same as above, but for macs	Not tested

Table 1: Code files for project

The following project files and folders are included for testing and troubleshooting

File or *Folder Name*	Use
Readme.pdf	This file. Overview of project
CellProfiler_RNAscope_3D.pdf	Manual for CellProfiler pipeline and Matlab code
R333 WT+3MC 40x 001_A01_G007_0001.oir	Test Image File for through processing
Project Code	All code for the Project (see table 1)
Image Files after FIJI Splitting	Files after running save_all_windows.ijm
Results From CellProfiler	Resulting csv files and images from CellProfiler
Results From Matlab	Resulting Spreadsheet from Cell_Profiler_3D_result_analysis.m

Table 2: Testing files and folders