**Colocalization Code README**

**Version Information:**

The code was created and tested on **Python version 3.11.4**.

**Required Packages:** numpy**,** math, os**,** csv**,** sys

These packages are essential for running the code and should be installed in your Python environment.

**Overview:**

The **Colocalization Code** collects statistics about the localization of mRNAs in smFISH images of neurons. The program is divided into two parts:

* **Part 1**: A semi-automatic tool for segmenting neuronal compartments (somas, nuclei, dendrites) and generating dendrite skeletons.
* **Part 2**: Uses spot coordinates from FISHQuant, dendrite skeletons, and prints from Part 1 to gather statistics about mRNA localization.

**Analysis Types in Part 2:**

1. mRNA density in cellular compartments.
2. Distribution of mRNA along dendrite skeletons.
3. Colocalization between two types of mRNA.
4. Colocalization of mRNA at synapses in dendrites.

**Updates in ARLIN v2.0:**

Three key updates were made in the second version of ARLIN:

1. **Improved mRNA simulation**: Better simulation of mRNA spots for more accurate computational controls.
2. **Additional details for colocalization and synapse localization analyses**:
   * Colocalization statistics now include spatial information by dendrite zone.
   * Synapse localization statistics have been refined to examine synapse serving by mRNAs in specific zones along dendrites.
3. **New functionalities**:
   * Synapse localization from the mRNA perspective.
   * Analysis of synapses being served by two mRNA species.

These changes primarily impact **Part 2** of the analysis pipeline, with modifications in part2.py and localization\_analysis.py.

**Zones in Dendrite Analysis:**

In ARLIN v2.0, dendrites are divided into "zones" of 25 μm each, starting from the soma and extending along the dendrite skeleton. The distance of each pixel from the soma is calculated, and the dendrite is divided into these zones for more granular analysis of mRNA localization.

**Part 1: Image Setup and Processing**

**1. Setting Up Your Folder of Images:**

* Create a dedicated folder for each experiment.
* Place all microscopy images (in .tif format) inside a subfolder.

**2. Naming Your Images:**

Ensure the following naming format for all images:

scss

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ExperimentName\_(DIV or div)##\_treatment\_listOfStains\_00#(xy or XY)##\_channel.gif

* DIV ##: Neuron age.
* treatment: Experimental conditions.
* channel: Microscope channel (e.g., Map2 for dendrites, DAPI for nuclei).

**3. Obtaining 2D Projections:**

Use **ImageJ (Fiji)** to generate 2D maximum intensity projections of .tif images:

1. Open the image in ImageJ.
2. Go to **Image > Stacks > Z Project** and select **Max Intensity**.
3. Save the output as a .gif.

**4. Making Annotations:**

To annotate dendrites and somas:

* Open the Map2 max projection .gif in an image editing software.
* Use the following colors to annotate up to 9 dendrites or somas:
  1. Red: RGB (255, 0, 0)
  2. Green: RGB (0, 255, 0)
  3. Blue: RGB (0, 0, 255)
  4. Orange: RGB (240, 134, 51)
  5. Yellow: RGB (255, 255, 0)
  6. Purple: RGB (143, 57, 182)
  7. Teal: RGB (130, 210, 208)
  8. Mint: RGB (214, 253, 208)
  9. Salmon: RGB (255, 128, 102)

**5. Running Part 1:**

Run part1.py to generate:

* **Outlines** for each mRNA channel.
* **Skeletons and Prints** folder containing skeletons and prints for each annotated compartment.

**Part 2: mRNA Localization Statistics**

When running part2.py, the command prompt will guide you through the following analysis options:

**1. Density Analysis:**

* **Input**: None.
* **Output**: Excel file (SomaDensities.xlsx/DendriteDensities.xlsx).
* **Statistics**: mRNA density per square nanometer in annotated compartments.

**2. Distribution Analysis:**

* **Input**: None.
* **Output**: Excel file (DistrAnalysis.xlsx).
* **Statistics**: mRNA distribution at various distances from the soma along dendrite skeletons.

**3. Colocalization Analysis:**

* **Input**: Two mRNA channels, maximum distance, and distance increment.
* **Output**: Excel file (ColocAnalysis.xlsx).
* **Statistics**: Minimum distance between mRNAs of type A and type B within dendrites, reported by dendrite zone (0-25 μm, 25-50 μm, etc.).

**Caption**: Output example showing colocalization statistics by dendrite zone for CY3 and CY5 mRNA, including a comparison to simulated data.

**4. Synapse Localization Analysis:**

* **Input**: Synapse channel name, threshold distance for localization.
* **Output**: Excel file (SynapseAnalysis.xlsx).
* **Statistics**: Number of mRNAs localized near synapses within each zone of the dendrite, reported by dendrite zone.

**ARLIN v2.0 Updates: Detailed Changes**

**1. Improved mRNA Simulation:**

In ARLIN v1.0, simulated mRNA was uniformly distributed along the dendrite. In ARLIN v2.0, simulated mRNA is now concentrated near the soma, reflecting the typical distribution of real mRNA. This is done by dividing the dendrite into "zones" and selecting simulated mRNA based on the number of real mRNAs present in each zone. This improvement provides a more accurate computational control when comparing real and simulated colocalization patterns.

**2. Additional Details in Localization Analyses:**

**a) Colocalization Analysis:**

In ARLIN v2.0, colocalization analysis is expanded to investigate where colocalization occurs along the dendrite. The program calculates colocalization statistics by dendrite zone, quantifying the proximity of mRNAs within each zone. Data is aggregated across all dendrites and zones, allowing trends to be visualized by zone.

**b) Synapse Localization Analysis:**

Synapse localization analysis has also been expanded in ARLIN v2.0 to include spatial information by dendrite zone. This enables investigation of where along the dendrite mRNAs are serving synapses, providing a more detailed view of mRNA-synapse interactions.