Investigation of stimulus-specific expression of multiple ITFs in different hippocampal cell types by smFISH data automatic analysis pipeline

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1 Introduction

Stimulus-dependent gene regulation is a widespread feature of biological systems and is important in excitable cells, especially in neurons. Responding to different patterns of activity could involve the expression of inducible transcription factors (ITFs) that drive subsequent gene regulation tailored to the initial stimulus. This process may underlie the formation of various skills and long-term memory [1, 2, 3].

For simultaneous registration of the expression of multiple ITFs, it is highly convenient to use the methods of fluorescence in situ hybridization. This makes it possible to evaluate the number of different transcripts in many cells using fluorescence microscopy, and novel RNA in situ hybridization (such as RNAscope) technologies allows simultaneous signal amplification and background suppression to achieve single-molecules visualization while preserving tissue morphology. As a result, the analysis of experimental data involves the processing of a large number of microscopic images, which is highly laborious for manual processing and prevents scaling.

2 Project Proposal

Due to the ever-increasing amount of experimental data in the form of microscopic images, manual processing becomes difficult. Automatic image analysis methods will increase processing efficiency and reduce the influence of the human factor, and the created analysis pipelines can be scaled for future experiments.

The goal of the project is to create a module for Python with a set of functions for analyzing images obtained by the smFISH method. Automatic RNA spots counting simultaneously for many genes of interest will be integrated with the segmentation of individual anatomical regions of the hippocampus, cell counting, and cell typing by expression of specific markers.
3 Timeline

The duration of the project is planned to be 12 weeks (July 11 - September 30). Supervision will be provided by Stefano Brigidi (University of Utah). The work plan and deliverables are provided below.

- **Week 1 (July 11 - July 15)**
  Reading papers and becoming familiar with a set of existing experimental data.

- **Week 2-5 (July 18 - August 12)**
  Implementation of methods for automatic segmentation of the anatomical regions of the hippocampus on images and detection of individual cells; development of a method for detecting RNA spots on images of various quality.

- **Week 6-9 (August 15 - September 9)**
  Implementation of detection and classification of excitatory and inhibitory neurons by specific markers and the introduction of the cell typing stage into the previously created pipeline.

- **Week 10-11 (September 12 - September 23)**
  Processing of preliminary experimental data with created pipeline and statistical analysis of results.

- **Week 12 (September 26 - September 30)**
  Completion of the project and writing documentation for the module, presentation of final results.

References

