

GeneExpress3D Quickstart Guide

Getting Started

Requirements

GeneExpress3D is a MATLAB GUI application and as such requires a working installation of MATLAB

Installation

Click the "Download Source" button above and extract the archive into a folder of your choosing. Start MATLAB and change its working directory to the folder in which you have extracted the downloaded archive. At the MATLAB prompt, type "GeneExpress3D" to begin!

Example Run

Loading data sets

On opening, GeneExpress3D displays four empty image windows on the right side of the GUI as well a set of processing buttons and settings on the left side. GeneExpress3D is designed to operate on three sets of data per experiment. The first data set is a nuclear stain, the second and third are fluorescent whole mount *in situ* hybridization (F-WMISH) data sets. To populate the GUI with data, select **Start New Analysis** from the **File** menu and load tif stacks of the appropriate data when prompted. If you wish to analyze a single channel F-WMISH data set, simple select the F-WMISH to be analyzed data twice. Example data sets are included in the download from github. Use nuc.tif for the nuclear data set with FISH.tif and FISH2.tif for the F-WMISH data sets.

Setting Analysis Parameters

Once the nucleus and FISH data sets are loaded into GeneExpress3D, the default settings for all analysis parameters are set for the GUI. The user must set the **X/Y Resolution** and **Z Resolution** for their data set. These values will be unique to each dataset depending on how the data was acquired. For **Threshold Level**, these settings are calculated for each data set. For all other parameters, the defaults are general settings that have yielded satisfactory results on a variety of data sets. The user may start with these settings and refine them later if desired.

Image Processing

A typical run through GeneExpress3D will next proceed with image thresholding, denoising, and dilation of the data sets in order to produce an overlap between the nuclear stain and F-WMISH signals. These steps are carried about by pressing the threshold, denoise, and dilate buttons as they become active on the left hand side of the GUI.

Nucleus identification and F-WMISH Expression assignment

A model of all nuclei in the data set is next constructed by pressing the **Find Nuclei** button. This constructs a three dimensional model of all nuclei from the the two dimensional stack of processed images in the nuclear stain data set. After this model is constructed, nuclei are assigned positive or negative F-WMISH expressions by pressing the **Find Positive Nuclei**

button. A summary table will appear with the calculated nuclear center positions as well as F-WMISH expression assignments for each.

Data Model Visualization and Exploration

GeneExpress3D provides a number of methods to visualize the outcome of the data analysis performed in the above steps under the **Tools** menu. In order to check the center positions of the identified nucleus the user may select **Check Nucleus Centers**. This tool brings up two windows. The first provides a maximum intensity projection of the nuclear data set with nuclear center estimates overlaid. The second window displays the nuclear data set with overlaid nuclear centers in a scrollable stack view. While exploring the images of positively labeled nuclei the user may find the visualization options under **Positive Nucleus Display Mode** helpful. The overlap percent cutoff for positive nucleus identification can be optimized using the **Check Overlap Percent Value** tool. Finally, the user may visualize the generated F-WMISH expression model in three dimensions by selecting **3D Plot of Final Data**.