Automated detection of transcription sites based on images with independent labeling of transcription sites

Overview

FISH-QUANT provides the possibility to automatically detect transcription sites based on an image with an independent label of transcriptions sites, e.g. LacI, intron FISH, or DNA FISH. We found this works best with filtered or deconvolved images since these images have reduced background making the detection of the transcription site easier.

Transcription sites are then found based on an intensity threshold. The user can also define the maximum number of sites that will be detected per cell. The program will then detect spots above this intensity background and if multiple are detected return only the brightest ones.

FISH-QUANT also provided an additional feature considering the fact that the LacI signal can be sometimes located away from the actual transcription site. The program will first look for the spots in the LacI channel. When this is done it will find for each spot the corresponding brightest voxel in the fish channel. This is restricted to a certain (user defined) area around the detected location in LacI. If the voxel in the FISH channel is above a certain threshold then the program will use the location of this voxel as the location of the transcription site, otherwise it will use the location defined by the LacI signal. This avoids corrections of the location if not FISH signal is present (no spot in the immediate vicinity of the LacI). So this intensity threshold should be set to a value corresponding at least to one transcript.

Basic workflow

Outline files

Outline files in FISH-QUANT allow associating not only the FISH image but also a DAPI image and an image with the transcription site label that can be used for the detection.

Determining the necessary parameters

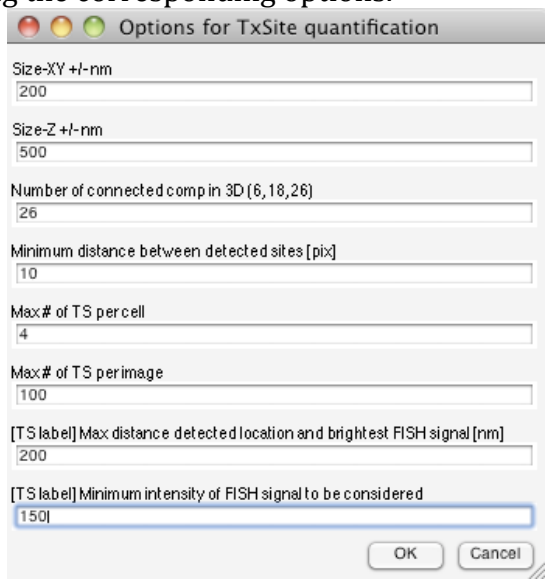
As described above there are a number of **parameters** that need to be properly defined for the detection to work. More information can also be found in the main help-file of FISH-QUANT. First, it is important to determine the expected intensities value. Here two values are important (a) what's the minimum intensity of a transcription site in Laci (b) what's the intensity of a typical spot in the FISH signal. Next, the maximum distance offset you observed between LacI and the actual FISH signal should be determined (expressed in nm).

Set-up automated detection

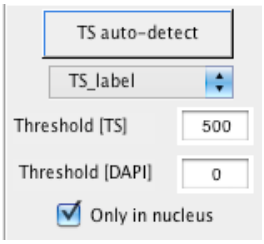
If these parameters are determined, the **automated detection** itself can be setup. This is done in the outline definition tool. First, load one of the outline files having the LacI images associated to them in the main interface. Then open the outline definition tool by clicking on the 'Define outlines' button. Confirm when FISH-QUANT asks you if you want to load the image with TS. In the interface the FISH and LacI image will be shown

together with the defined outline(s). The contrast and transparency of each image can be changed with the corresponding controls. It is also possible to shown only show one image with or without the outline by enabling the corresponding options.

First, the **general settings describing the detection** including the parameters to define the allowed offset between LacI and FISH images are define. The corresponding dialog box can be opened from the menu Options > TxSite detection. The different fields are explained in more detail in the FISH-QUANT help file. The most relevant ones are the last two. Here, the allowed maximum distance between the identify LacI and the FISH signal is specified (200nm in the example on the right), as well as the minimum intensity the FISH signal has to have to be considered (150 in the example shown on the right).



Then the **intensity threshold to detect the LacI signal** is specified directly in the outline interface. This is done in the outline gui directly in the panel for the TS detection. The corresponding controls are shown on the right.



First and **IMPORTANT**, select in the pull-down menu 'TS_label' to tell FISH-QUANT to use the LacI image for the detection. The defined the intensity threshold determined for the LacI signal in the field "Min. intensity". The other fields are optional and further restrict the detection. For each cell a nucleus can be defined, if such a nucleus is defined the detection is restricted to the nucleus. Alternatively, an image with a DAPI signal can be loaded and an additional intensity threshold for the DAPI signal can be set. DAPI is much brighter in the nucleus so this usually restricts the detection also to the nucleus. Then the settings can be **applied** by pressing 'TS auto-detect'. The detected transcription sites in each cell will then be displayed. The detection settings have to be **saved** from the menu *[FQ outline] > Save settings for TS detection*. These settings can then be used in the batch processing tool to detect sites in all cells.

Automated detection in batch mode

1. Define the folders for images and outlines.
2. Load the settings of the mature mRNA detection. This is necessary to get the general experimental parameters.
3. Load the outline files.
4. The automated detection results in new outline files which will be saved in the defined folder for the outlines. To separate these outlines from the original ones, we

recommend changing the folder for the outlines before performing the automated detection.

5. Load the transcription site detection settings with the button ‘Load setting’. This will load all the settings specified in the outline tool. The user can still change the intensity threshold used for the LacI signal. This value is shown in the text box ‘Intensity threshold’.
6. Perform the automated detection by pressing ‘Detect TS’. This will go over the specified outline files, find automatically the transcription sites, and save the new outline files.
7. The automatically generated outline files can then be inspected with the FISH-QUANT directory browser. See section ‘Inspection of directory content’ in main help-file. In short, the outlines files can be opened from a simple browser window and inspected. If necessary corrections can be made and saved.
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