FISH-quant - version history

Version 2e: pre-release

New features / improvements

Corrected bugs

Version 2d: September 19, 2014

New features / improvements

Outlines files. During batch processing new outline files are created (mature RNA detection and automated TS detection). These are now saved in a SUB folder of the fodler where the origina outline files can be found.

Pre-detection. The pre-detection method can now be chosen already in the dialog box that opens before the pre-detection GUI is shown.

Spot inspector. Labels of all cells can now be displayed. It's also possible to show the outlines of all cells and not only of the cell where the detected spots are displayed.

Batch processing. It is now possible to load filtered images with user defined replacement strings. This allows automatically loading image from other software packages as filtererd images, e.g. deconvolved images.

Transcription site quantification. Interface for TS quantification and corresponding part in batch interface have been simplified. By default only the quantification with the integrated intensity is proposed. Only if specified the more complicated controls needed for the PSF superposition approach are shown.

Corrected bugs

Spot inspector. Corrected figure legend when showing detected and thresholded spots (before colors were inversed).

Version 2c: March 10, 2014

New features

Mature mRNA detection: FISH-quant now also reports the area of the cell and (if defined) nucleus in the summary file of the mature mRNA detection that can be created from the batch module.

Folders can be saved in a text file and loaded later.

Spot inspector: you can now choose if detected spots are shown or not.

Outlines tool allows now faster loading of DAPI and TS-label images. This option is useful when outlines for many images are defined. When the first time a DAPI or TS-label image is opened the unique identifier for these images has to be defined, e.g. _DAPI_ and _FISH_. FQ will then look in the file-name of the FISH image for the identifier for the FISH and replace it with the identifier for the DAPI. This name will be proposed as a default name. Same approach is used for TS-label. Further, short-cuts have been defined to load images. A further speed-up can be achieved when combined with the 'List directory content' tool. Here you can navigate to the folder containing the images. When clicking on an image, it will be automatically opened in the outline designer. For the first image, the default names have to be specified. For the subsequent, the will be remembered.

New GUI to process multiple folders. Allows having different settings files in each folder.

Corrected bugs

- Show filtered image from pre-detection GUI works now.
- Main interface: loading new image did not reset the selection to show detected spots in interface. This is corrected now.
- The automated assignment of transcription sites to cells did not always work. Specifically, the TS of the currently selected cell where attributed also to the cell where the new TS was drawn. This is now corrected.
- Outline-designer: zoom is reset when new image is loaded.

Version 2b: September 25, 2013

New features

Mature mRNA detection.
Detection can now be performed either for the entire cell, only the nucleus, or only the cytoplasm. Requires that the outlines of nuclei are defined. Implemented based on a suggestion of Gal Haimovich.

Outline definition.
Before the cell had to be selected in which a TS or the nucleus is outlined. Now these outlines can be drawn over any cell and FISH-quant automatically determines the corresponding cell. Implemented after discussions with Gal Haimovich and Ronit Salomon-Kent.

Spot inspector.
Index of each spot can now be displayed next to the spot. Implemented based on a suggestion of Gal Haimovich.

Batch module
Auto-save option is now also available for mature mRNA detection. Implemented based on a suggestion of Gal Haimovich.

Batch module
New menu item to reset the interface to restart analysis (*[FQ] batch > new analysis*). Now the list of outline files can be changed (files added, deleted) but these controls are disabled as soon as the processing starts. In order to add new files, a new analysis has to be started.

Filtering
- For certain combinations of pixel-value and z-stack size the filtering resulted in an image with no distinguishable spots. Now the Kernel size is determined directly in pixels which solved this problem.

- Kernel-size can also be defined independently for XY and Z. We found that for images with only a few z-slices using a small Kernel in Z for the background filtering (around 1) yields a better description of the background and reduces the number of false-positive detections.

32 bit
FQ now also supports 32 bit TIFF images.

Corrected bugs

- **Batch module**. Error message when loading analysis results from .mat file and attempting to save results of nascent mRNA quantification.

- **TxSite quantification**. Problems occurred when analyzing TxSites corresponding only to background. Such sites can be detected with an independent label such as LacI or by using FISH probes against different parts of the gene, e.g. 5'vs 3'probes. Fitting of such background signal with a Gaussian function can lead to large sigma's. This can then give erroneously large estimates for nascent mRNA. These fits are now automatically detected and the corresponding values for the number of nascent mRNA set to 0. This affects only the methods based on the fits and not the superposition approach.

- **Main interface**. Reset GUI is now working.

Version 2a: March 25, 2013

Initial release accompanying publication in Nature methods.