NuFoQu

Yeast foci analysis from 3D fluorescence microscopy images

Created by Mickaël Garnier (mickael.garnier@curie.fr) **on June 15, 2021**
Following the work done by the PICT@Pasteur and the Taddei lab

Last edit August 4, 2025

Script purpose:
The NuFoQu matlab script performs quantitative analysis of foci in different yeast strains and/or conditions. A few parameters allow the users to target specific spots inside the nuclei and adapt to their image quality and specificities. A second step can be used to filter out spots and nuclei that are badly segmented or should not be included in the following analysis. Finally NuFoQu can generate descriptive graphs to represent the data.

PROTOCOL

Step 1 [Time required 5 minutes]

Organise your files and data

- The **NuFoQu.m** file and its dependencies (**institut_curie.png** and the **utilities** folder) should all three be in a single directory
- Prepare you image data:
  - Have all your images inside one or two folder (for deconvolved images)
  - The image names in both folders should be the same
  - The yeast strain and any relevant condition should appear in the image names

Step 2 [Time required 2 minutes]

Start the NuFoQu script

- Run MATLAB
- Start NuFoQu, either:
  - Click on the top right NuFoQu shortcut (if present)
  - Go to the NuFoQu folder and type NuFoQu in the command window
  - Click the run ▶ button or hit F5 from the NuFoQu file in the MATLAB editor window
- If asked, select the option: **Change Folder**
- The user interface should appear shortly after

Step 3 [Time required 5 minutes]

Set parameters

- Choose wether you want to analyse a single file or a complete folder
- The option **Segmentation on two** will have you select two folders (or images):
  - first for quantification, usually the raw data
  - then for segmentation support, usually deconvolved images
- Check the **Save segmented images** if you want to be able to supervise the segmentation results
- Click the **Choose data location** button, a file explorer will open to select the image/folder to analyse

DETAILS

Operating System
Tested on Debian/Ubuntu, Windows 7/10 and Mac OS 10.15

MATLAB
Requires version 2020b or newer

Bugs
Report bugs to contact author

NOTES

- Click the **Choose results location** button, a file explorer will open to select the folder in which all the quantifications, segmented images (if necessary) and graphs will be written
- Click the **Set parameters** button to open another window to enter the algorithm parameters:

  XY pixel size (μm) height and with of a pixel in micrometers

  Z pixel size (μm) depth of a voxel in micrometers

  Foci size (pixels) approximate dimension of the targeted foci

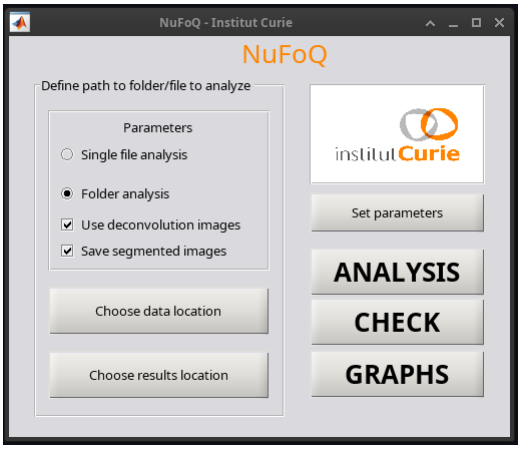
  Z-slices to skip number of Z slices from the top of the stack to remove from the analysis

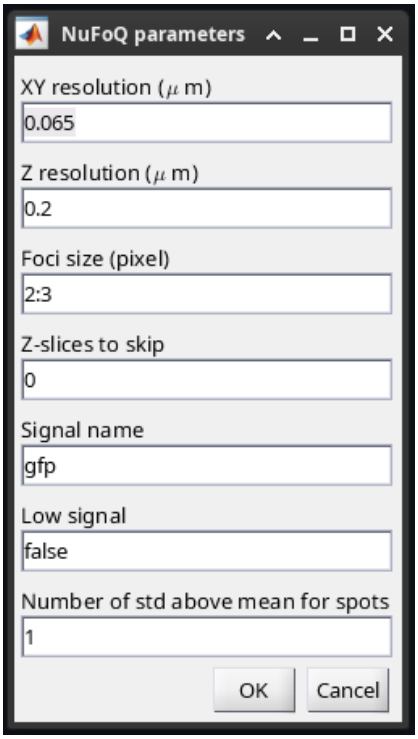
  Signal name indicator of the channel to quantify, the script will look for these characters in the file names to perform the analysis

  Low signal indicator to perform a variation on the algorithm in case of really low signal to noise ratio. Try without and, if needed, try again with this option set to **true**

  Number of std above the mean for spots Filter to remove spots with peak intensity lower than the average of their nuclei plus a certain number of the nuclei intensity standard deviation. This value can be any rational number

  Number of pixels to add on nuclei contours Dilate the nucleus masks by a sphere of the specified radius
- Click the **OK** button





Step 4 [Time required ∞ minutes]

Run the analysis

- Click the **ANALYSIS** button
- Wait for the function to finish running

Step 5 [Time required ∞ minutes]

Check and supervise segmentation outputs

- Click the **CHECK** button


qFOCI 2.0 - Institut Curie - Supervised filtering of analysis outputs
Display settings
Minimum
Sum
Maximum
Mean
Std deviation
Median
Contrast
Min
Max
Overlay
Off
On
Colormap
gray
Data
Nuclei kept
Nuclei killed
Spots kept
Spots killed
Nuclei
Keep & Kill
institutCurie
Next / End

- Select a **.spots** file to supervise
- Wait for the interface to load (this may take a few minutes)
- Set the visualisation parameters

  Type of Z projection Choose the computation method to render a 2D image

  Contrast Set the min and max gray levels on display

  Overlay Toggle the green/red overlay on top the image

  Colormap Replace the gray levels with the chosen fake colors
- Remove/restore spot and nucleus masks, killed objects are removed from further analysis

  Lists List the labels of all the nuclei/spots that are kept and killed

  Nuclei/Spots Switch between nuclei or spots selection

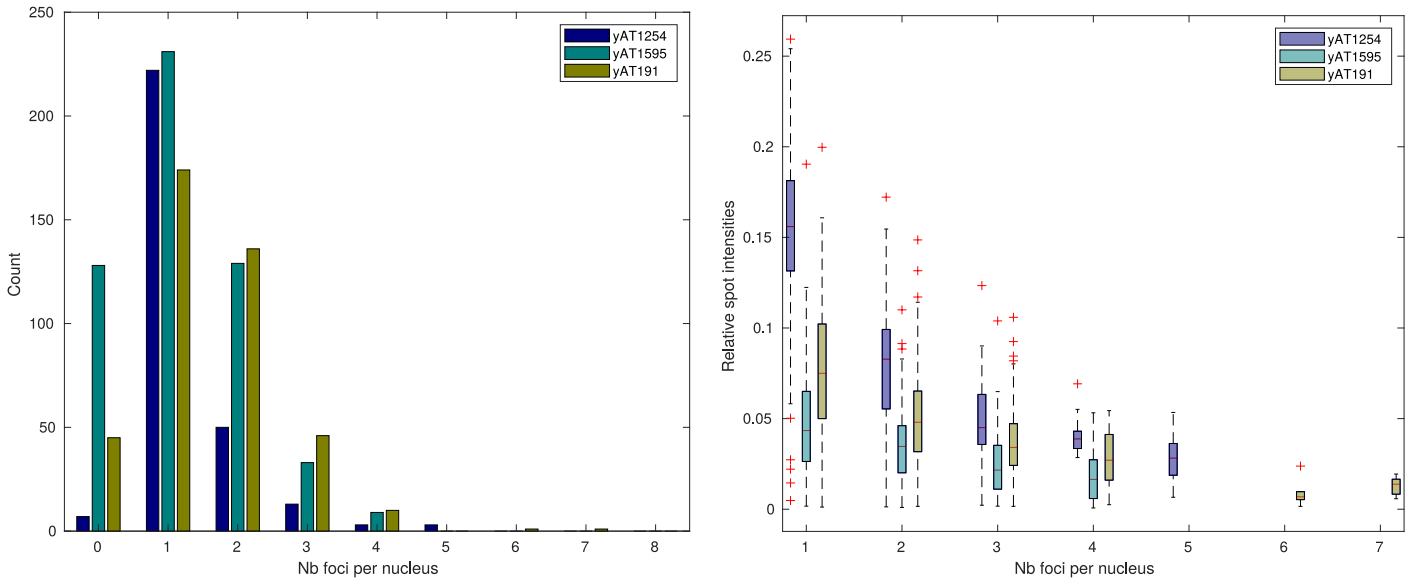
  Keep & Kill Move the selected object in the other Kept/Killed list
- Select an object by clicking on it in the image window or on its label in any list
- Selected objects appear in yellow for nuclei and pink for spots
- Click the **End** button to save the modifications and close this window
- All modifications performed here are flags in the quantification files and thus everything can be reversed

---

Step 6 [Time required 3 minutes]

Generate descriptive graphs

- Click the **GRAPHS** button
- Select the **.spots** (generate during the analysis) files you want to include in your graphs
- Select (or create) the folder in which to write the graphs and the exhaustive quantification table (**all_measurements.txt**)



Examples of two outputs graphs. Left **histograms.pdf** and right **grouped_boxplot_spot_relative_intensities.pdf**

QUANTIFICATIONS

Nuclei measurements:

- **Nucleus intensity** : Total intensity inside nucleus
- **Nucleus background** : Sum of intensity inside nucleus without spot contents
- **Nucleus volume** : Volume of nucleus (in pixel^3)
- **Nucleus spot number** : Number of spots inside nucleus
- **To keep** : Flag indicating to preserve or remove nucleus in further analysis

Spots measurements:

- **X** : X coordinate of foci
- **Y** : Y coordinate of foci
- **Z** : Z coordinate of foci
- **Volume** : Volume of foci (in pixel^3)
- **Intensity** : Total intensity inside foci
- **Relative intensity** : Ratio of nucleus intensity inside foci
- **Distance to border** : Distance between the foci centroid and the closest nucleus border (in pixel)
- **Relative distance to border** : Distance between the foci centroid and the closest nucleus border normalised by nucleus equivalent radius
- **Nucleus ID** : Label of nucleus
- **Nucleus intensity** : Total intensity inside nucleus
- **Nucleus background** : Sum of intensity inside nucleus without spot contents
- **Nucleus volume** : Volume of nucleus (in pixel^3)
- **Nucleus spot number** : Number of spots inside nucleus
- **To keep** : Flag indicating to preserve or remove foci in further analysis
- **Elongation** : Number indicating the elongation of the segmented foci. Currently experimental

OUTPUT FILES

Step 1 — Analysis

***filename*.nucs file**
Quantifications of nuclei

***filename*.spots file**
Quantifications of foci, includes their respective nuclei measurements

Optional:

***filename*_segNuc.tif**
Segmentation mask of nuclei, with labels

***filename*_segSpots.tif**
Segmentation mask of foci, with labels

Step 2 — Supervised check

***filename*.nucs file**
Updates the **To keep** flag

***filename*.spots file**
Updates the **To keep** flag

Step 3 — Descriptive graphs

all_measurements.txt
Exhaustive table including all quantifications with image name and unique labels for foci and nuclei

boxplot_*quantification*.pdf
Boxplot and grouped boxplot of nuclei and spots measurements

histograms.pdf
Grouped histogram of number of foci per nucleus for all yeast strains

***strain_quantification*.pdf**
Probability density function of a specific quantification for a single strain per number of foci per nucleus

***strain*_histogram.pdf**
Histogram of number of foci per nucleus for a single strain