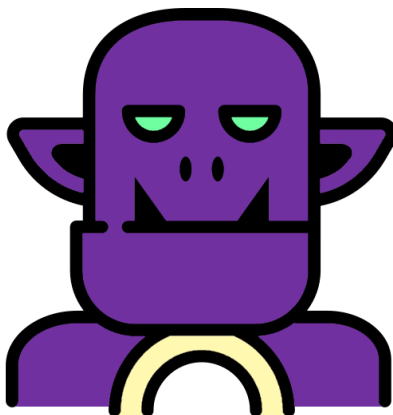


# EASI-ORC Guide:

(Shahar Garin, August 2023)



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## Introduction

This document aims to thoroughly explain the different steps required for fluorescent image analysis using EASI-ORC and make them accessible with relatively little know-how. It requires basic knowledge of working with computers, and how to activate scripts in Fiji (Plugins → Macros → Run...).

Prerequisites, such as file formats or needed values are explained in each section, so please read the instructions carefully.

Examples shown in this guide were mainly based on ER segmentation, but the processes described in it can also be used for any other organelle you wish.

The guide and scripts were all written and used while using a windows PC. Use of any of the scripts on different systems may create issues.

All scripts' code can be found in the [EASI-ORC GitHub](#). Each module is described with the steps taken by the corresponding script. These descriptions can be used to perform the steps manually, through FIJI's menus, if you wish to check each step's effect on your images yourself.

### **Applications you need to install before beginning:**

Fiji can be downloaded for free [here](#).

YeastMate standalone app can be downloaded for free [here](#) (download .exe file)

YeastMate plugin Fiji installation can be performed by following instructions on [this page](#).

RS-FISH plugin Fiji installation can be performed by following instructions on [this page](#).

## 1. Requirements and Folder Structure

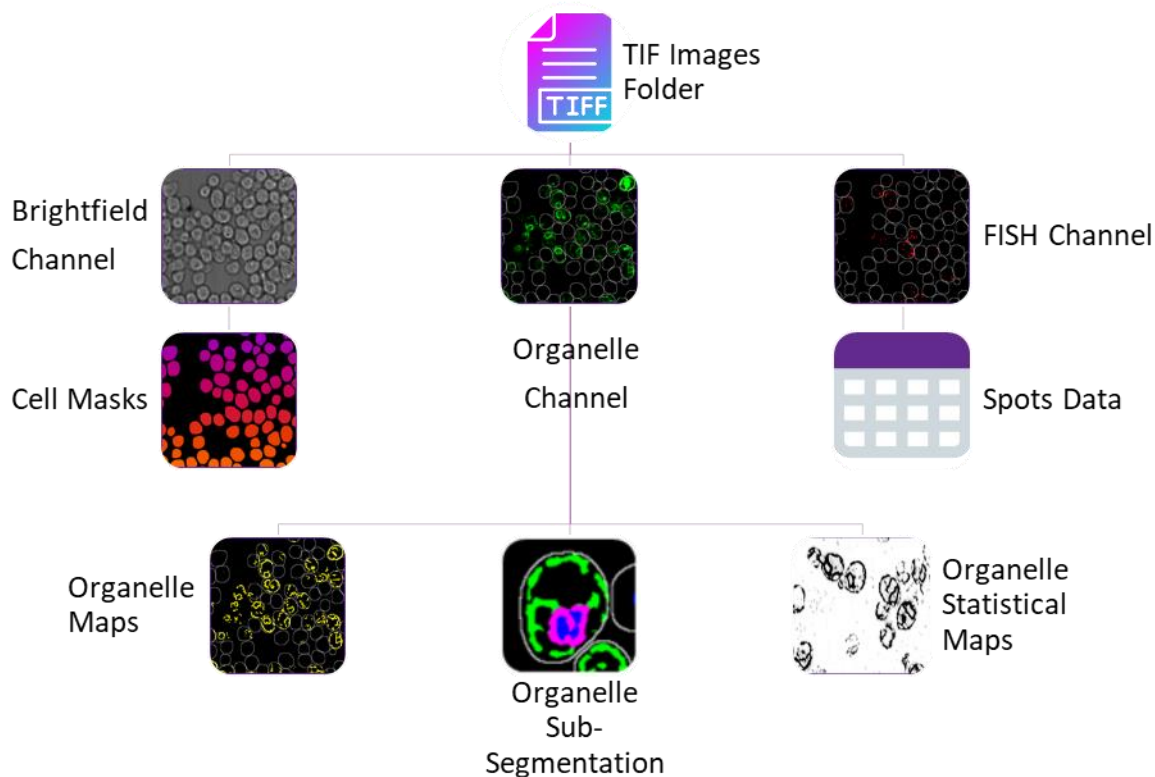
By and large, EASI-ORC can work on any modern system. It will work faster, the better the CPU, but it can complete its work without needing state of the art hardware. However, it is limited by RAM size. The higher the image resolution, the more RAM is required. The system was tested using 2048x2048 images and for that resolution we would recommend at least 32 GB of RAM. Anything less can result in some of the steps (specifically cell and organelle segmentation) crashing midway.

Another requirement concerns the way you capture your images. smFISH signals are identified using data from multiple planes of capture. As such, making sure your fields are correctly focused

is important to maximize your yield of usable cells. Specifically, signals should be least focused on the edge z planes and most at the middle of the range.

Also, EASI-ORC relies on a specific folder structure. Every image of each channel captured must be saved in a separate folder, with nothing but images in it. The images should be in the TIF format. In the next segment we include a script that can convert the images to TIF and order them in folders according to channel. Lastly, high resolution images require a large amount of space. Before running EASI-ORC, make sure you have available space that is at least 3 times that of your raw images. After completing the scripts run and image analysis, you can remove any images you wish, unless you plan to repeat the colocalization analysis.

The final folder structure created by EASI-ORC is presented in the figure below.



## 2. How to use a Fiji Script:

Each script is a single 'ijm' file. To use it, simply open Fiji and drag the file to the Fiji menu widow. A new window will open, containing the script's code. Press the 'Start' button (below the code section of the window) and the script will run. Any inputs needed will appear in pop-up windows.

### 3. Convert Raw Images to TIF and Arrange in Folders:

Most microscope software outputs images in a format unique to the manufacturer. These file types (*i.e.* bio-formats) can be less accessible for many programs or processes. Accordingly, we must make sure all files are in the correct format.

This script assumes each channel and scene are saved in a separate image file (**no multi-channel images**). File names must be unique for each channel. It can have the name of the marker (GFP, DAPI, mCherry, etc.) or an arbitrary name (c1, c2, etc.). The user must know these names and input them (**case sensitive**) once he runs the script.

**Important** – if your files are formatted differently (in a multi-channel image, for example) this script won't work. You must first convert each channel to a separate image. You must have each channel's images in a separate folder, in TIF format, before using the EASI-ORC scripts.

- 1) The user will be asked to input the directory containing the images and their file type (usually appears at the end of the file name, after a dot; tested with 'stk' files).
- 2) The user will be asked to input the number of channels, as well as each channel's name. Channel names must be the same as they appear in the corresponding file's name (**case sensitive**) and they must be unique per channel.
- 3) Each raw image will be opened sequentially and saved in its proper folder as a 'tiff' file, with its original name.
- 4) The 'tiff' images will be saved in a new folder located in the same location as the raw images. The main folder is called 'Tiff Images' and sub-folders for each of the channels will be created in it.

### 4. Use YeastMate to Segment Yeast Cells in an Image:

One of the first challenges in image analysis is identifying and defining each cell in the image. When it comes to images of yeast cells, YeastMate is a Fiji plugin that provides fast and trustworthy results, using a pre-trained, machine learning approach. The script uses the app's default settings, with a change to the threshold value to 0.6, to maximize the number identified cells.

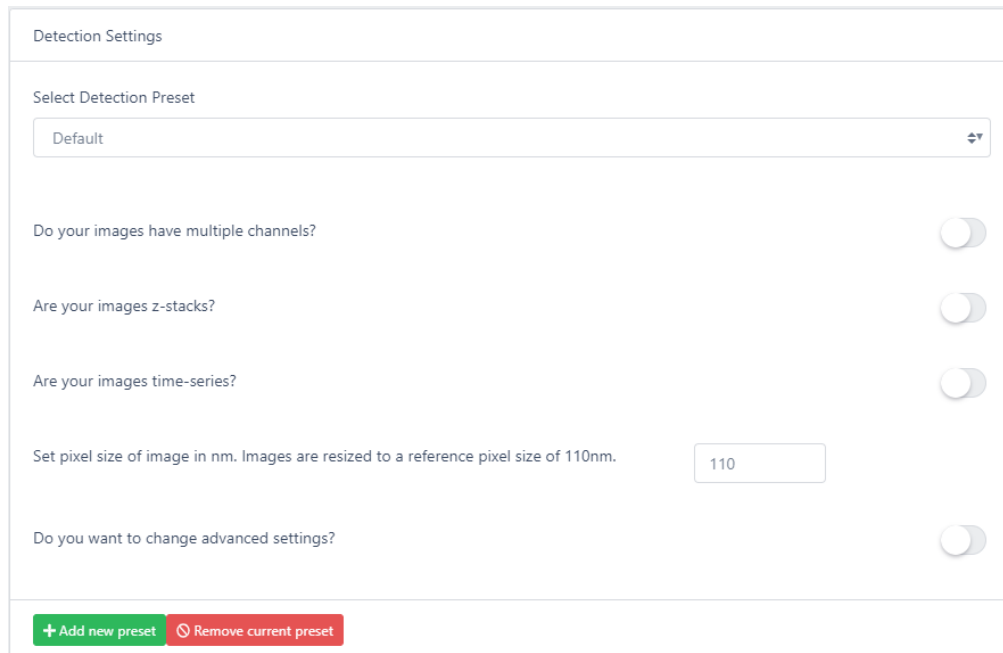
Crucially, to use the YeastMate plugin, you must install its standalone application. Failing that, the script described here will not work. The plugin itself must also be installed to Fiji. See the introduction for links to download the app and the plugin.

To choose the best plane from a multiplane image, masks of each plane will be created and the one containing the largest number of cells will be chosen for the final image.

In addition, **YeastMate must run on a computer without interruption**. It can take a long time to get a mask image when using high resolution images with many z planes. If you have many images to process, take that into consideration. For example, when processing 50 images with 12 slices each, at 2048x2048 resolution, the script needed ~4 hours to complete. Timing will vary greatly according to available computing resources and image size.

Once you install the YeastMate application and Fiji plugin (instructions for installation available here), you can start the application to get ready to run the EASI-ORC module. Follow these steps:

- 1) The program will start in 'Start a new job' tab (this and the following stage can be performed before you run the script).
- 2) Press the 'Detection' button under settings. Your settings should be all be turned off, as below:



Detection Settings

Select Detection Preset

Default

Do your images have multiple channels?

Are your images z-stacks?

Are your images time-series?

Set pixel size of image in nm. Images are resized to a reference pixel size of 110nm.

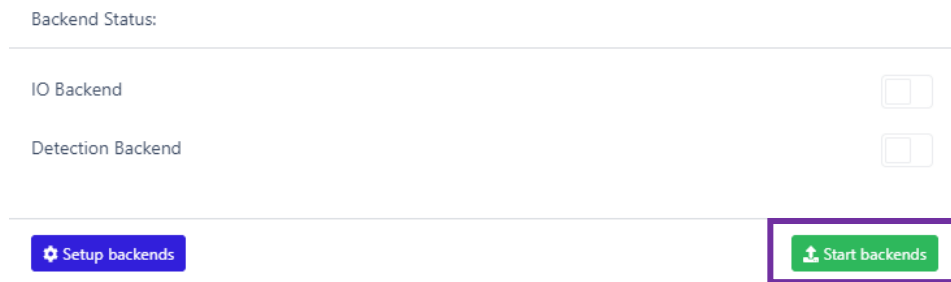
110

Do you want to change advanced settings?

+ Add new preset - Remove current preset

You may change the default pixel size according to your specific image parameters, but if it's not significantly different, YeastMate will work well.

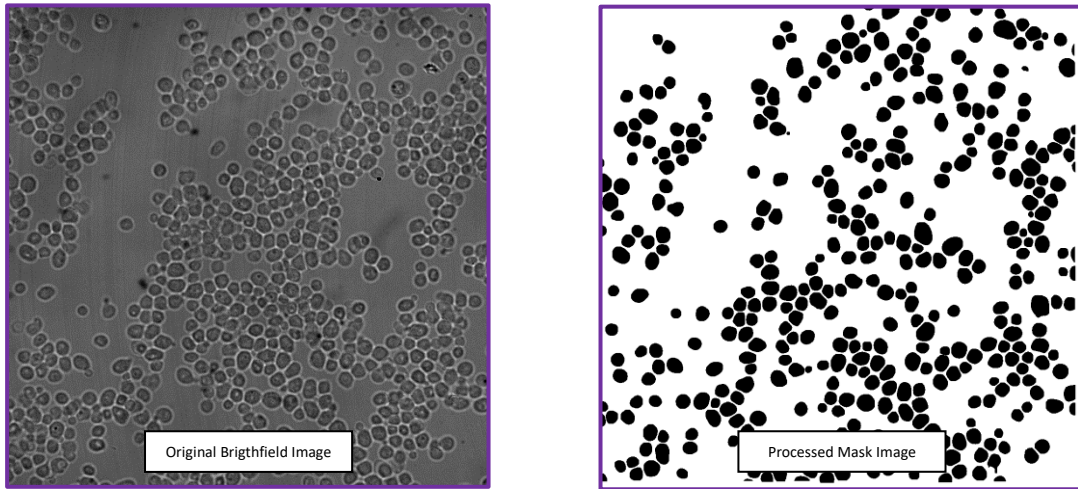
- 3) Back in the 'Start new job' page, press the 'Start Backends' Button. Two console windows will open. Both IO Backend and Detection Backend markers will turn green.



### Script Steps:

- 1) First, the user is prompted to choose the folder with the cell images in visible light (brightfield or DIC).
- 2) The user is asked to make sure they opened YeastMate. After making sure YeastMate is ready (backends are running), and clicking 'OK', the script will continue.
- 3) For each image in the folder:
  - I. Image is opened.
  - II. For each slice:
  - III. YeastMate will create a multi-colored mask image. Masks will be transformed to binary images.
  - IV. Using Analyze → Analyze Particles, cells are added to the Roi Manager and counted.
- 4) The plane with the largest number of identified cells is chosen.
- 5) The chosen plane is duplicated: Image → Duplicate n=number of original planes.
- 6) The images are converted to binary images: Process → Binary → Convert to Mask.
- 7) A new, stacked binary image is created: Image → Stacks → Images to Stack.
- 8) The image is saved in the same folder as the original images, under the 'Cells Masks' folder.

For each image you will get a binary image, with a white background and the shape of the cells in black. This will be used downstream to identify each cell's location.



**Unsolved Bugs or issues:**

Low quality images, where no cells are identified may occasionally lead to YeastMate crushing for one or more of the slices, resulting in the script crashing as well. In such a case the image cannot be used and must be removed from all folders (*i.e.* all channel images) before running the script again. Also, before re-running the script, make sure to delete all the folders added in its disrupted run, to avoid any clashes.

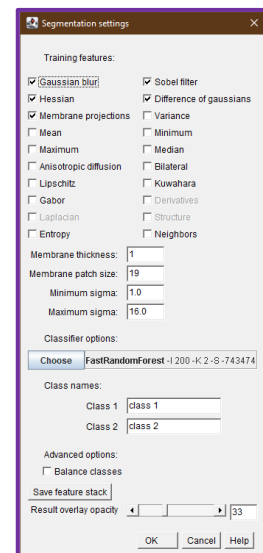
Once the script is done, the Fiji 'Console' window may open, presenting an error. It can be ignored.

## 5. Use Trainable Weka Segmentation to Train a Model for Sub-cellular Segmentation:

Identifying and defining sub-cellular structures is a more challenging step, the less regular the structure's shape. In the case of ER, for example, its shape varies considerably from cell to cell. The solution from Weka, in the form of a Fiji plugin, allows a user to train a model easily, and using the results of the training (a file called a classifier or a model) to perform segmentations of all similar signals in many different images. Here follows a detailed explanation of the training process.

### Classifier Training Steps:

- 1) Open an image of the appropriate channel in Fiji.
- 2) If you wish to save time, make sure to <sup>3</sup> only use a single z plane image for this stage (in a multiple stacks image: Image → Stacks → Stacks to images and select a z plane window with a good signal).
- 3) Press Plugins → Segmentation → Trainable Weka Segmentation. The pictured window will open.
- 4) To train the classifier, you must mark specific pixels of each class (your organelle, background etc.). Name each class by pressing 'Settings', at the bottom left. The pictured window will open.
- 5) Change class names to fit your experiment (for example, 'ER' and 'Background').
- 6) Under the settings window, you may also change different features that can alter the behavior of the classifier. Notice adding more training features will result in longer training and identification times, and require more computational resources, but feel free to experiment. The settings in the picture are the ones used by us.

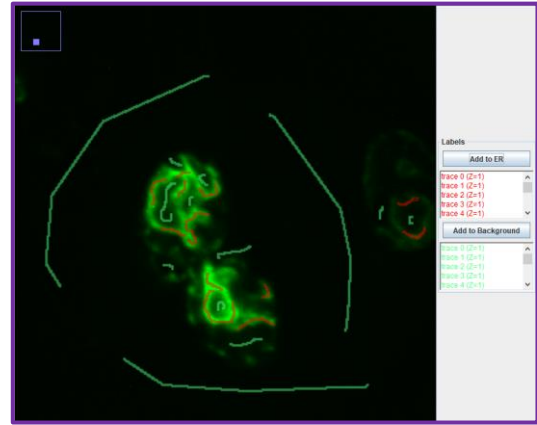




7) Now you can mark different classes on the image using the mouse. It is recommended to adjust brightness/saturation and zoom in for this stage (adjusting brightness won't affect the classifier).

8) Mark a small area of the image each time, and press 'Add to Class' button. The markings will change color and be added to the list under the class name (pictured).

9) Avoid adding a marking to a class, if the marking is inaccurate. You cannot remove a marking once it's added. You can start marking without saving, and the previous marking will disappear.



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10) After marking and adding a few times for each class (no need to add more than 5-6), press 'Train classifier'.

11) After training will end, you will be able to see how the different classes are marked on the entire image (pictured).



12) Save the classifier by pressing 'Save classifier'.

13) Repeat these steps for several different images (use the 'Load classifier' button and select the classifier you created). The more you use, the better. Only a few marks in each image are enough.

14) You can test and re-train your classifier on different images of the same channel/signal. By pressing 'Get probability', you can look at a probability map resulting from your classifier. Make sure to look at it next to the original image and make sure the signals match.

## 6. Perform Sub-cellular Segmentation Using a Trained Model:

The following script uses a trained classifier on multiple images in a user input folder.

The script assumes a classifier was already created (as described above), and the input folder contains only images of a channel corresponding with the chosen classifier.

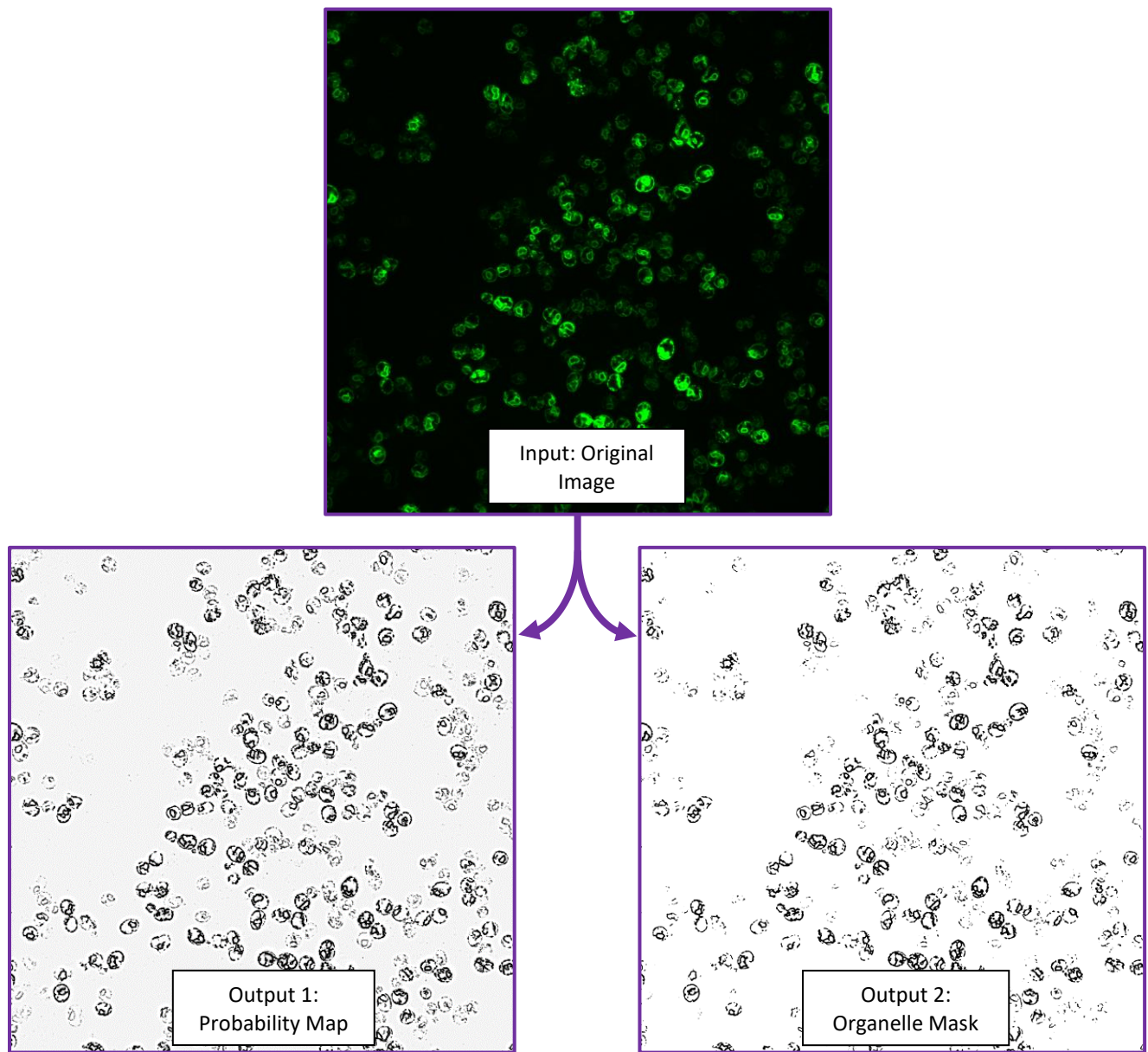
The script saves both the organelle mask and statistical map files. The statistical maps are not used downstream by other scripts but can be important for troubleshooting. After going over a few of them to make sure organelle segmentation was performed well, they can be deleted.

This step is very time consuming. When using a classifier for the first time, it is recommended to test it by using the script on a few images (3-4) and examine the resulting masks, to make sure the results are of high quality.

### Script Steps:

- 1) The user is asked to input the folder containing images of the channel they wish to segment and the classifier (through a browse window).
- 2) Subfolders are created to save the probability maps and mask images, in the input folder.
- 3) For each image:
  - I. Each z plane is saved as a separate file (in a temporary folder: 'seperated\_planes').
  - II. every separate z plane image is opened, one at a time, and the following actions are performed:
    - i. Run Trainable Weka Segmentation plugin.
    - ii. Load classifier.
    - iii. Create a probability map.
    - iv. Save probability map in subfolder (in a temporary folder: 'temp\_maps').
    - v. Close all.
  - III. After all planes from one image have a probability map, open them all.
  - IV. Stack to a single image (under the same name + '\_map').
  - V. Save probability map image with multiple z planes in probability maps folder.
  - VI. Create a mask file based on the probability map (using IsoData thresholding) and save it.
  - VII. Close all.

VIII. Delete temporary image files from 'seperated\_planes' and 'temp\_maps' folders.



## 7. Organelle Sub-segmentation:

ER is usually divided to cortical (cER) and perinuclear (nER) ER. The first is proximal to the nuclear membrane, and the second is distal to it. When checking for mRNA colocalization with the ER, it is common to calculate it separately per sub-section. EASI-ORC can separate the two sub-sections, by using an image of the nucleus as a proximity marker. The nucleus is a specific case of a proximity marker that allows the sub-segmentation of an organelle. The process can be performed with any other proximity marker you choose, to sub-segment any signal you've previously made a mask for.

This script assumes the Trainable Weka Segmentation process was performed on both organelle and proximity marker signals and masks for each exist. Each must be saved in their own folder (without any subfolders) and the files must be ordered in the same manner. If the files were created using the scripts in this guide, all these conditions are met.

**Important note:** In cells or z planes without a proximity marker signal, there will be no way of identifying the sub-sections of the organelle that are close or far from the marker. As such, these cells, in these planes, must be filtered out in the colocalization calculations (the EASI-ORC colocalization script takes that into account).

### Script Steps:

- 1) The user is asked for the folder of the proximity marker and organelle masks.
- 2) The user is asked for the number of dilation steps (adding 1 row of pixels near signal edges) to be performed on the proximity marker signal.

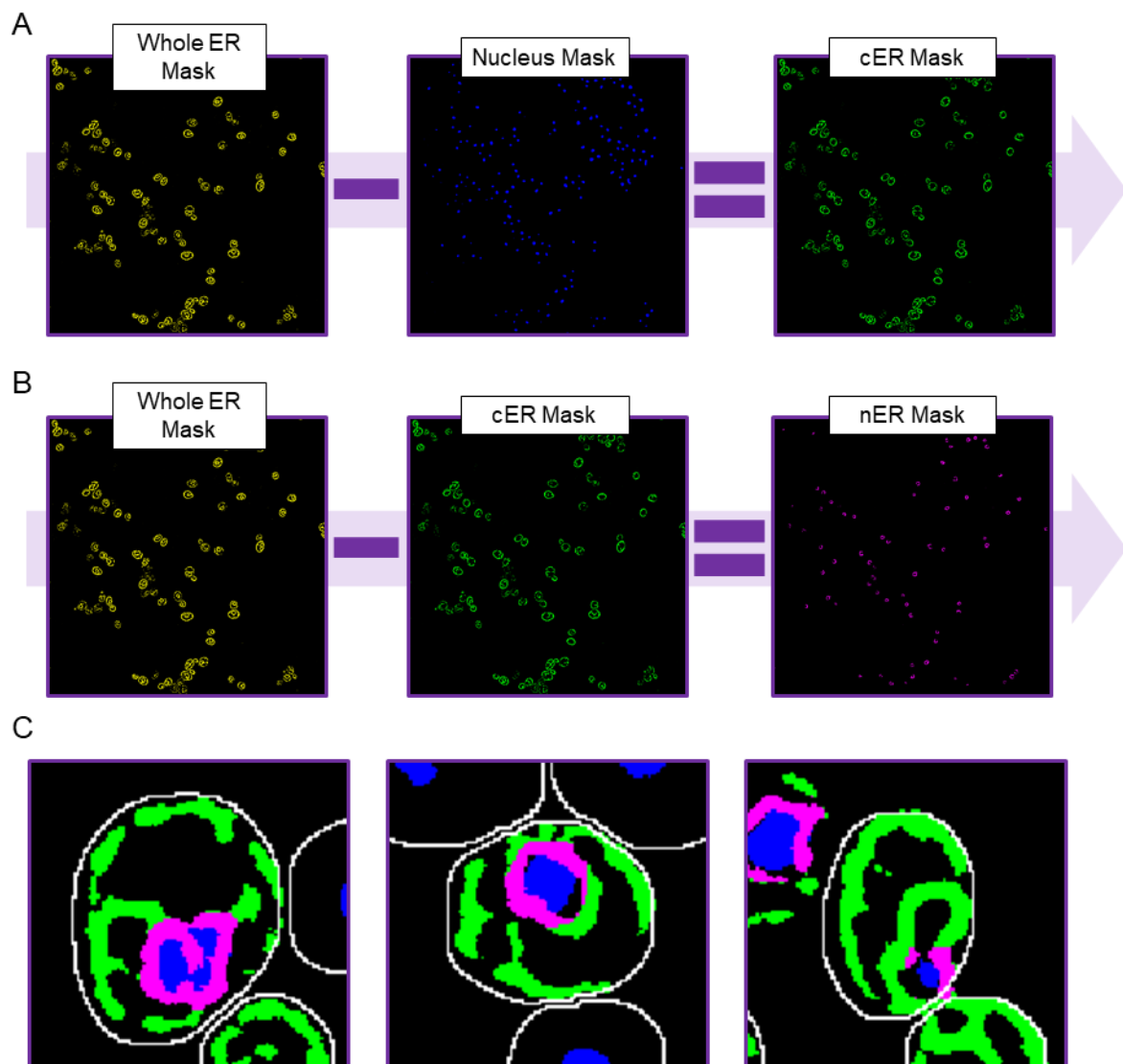
**\*Note:** It is recommended the user tests this manually with several values, to make sure the proximity marker is of satisfactory size to cover the proximal sub-section (for ER sub-segmentation using a nuclear marker, we found 3 steps works well). To check for yourself, simply open a proximity marker mask and go to the menu: Process → Binary → Dilate. You can then merge the proximity marker image with the organelle image and see if the coverage is sufficient.

- 3) For each proximity marker image:

- I. Process → Image Calculator... Performs subtraction of the proximity marker mask image from the organelle mask image, outputting and saving a mask image containing only the distal sub-section.
- II. Process → Image Calculator... Performs subtraction of the produced distal sub-section mask image from the original organelle image, outputting and saving a mask image containing only the proximal sub-section.

All images are saved in the original organelle mask folder.

The process and the effect of different qualities of proximal markers are shown below.



## 8. Manual run of RS-FISH for threshold identification:

smFISH signals are relatively simple signals, that can be identified using specific plugins. RS-FISH, is one such plugin. It's fast, accurate and considers signal intensity between different z planes. Crucially, it can be automated using Fiji scripts.

**Important:** To properly identify FISH spots, RS-FISH requires a signal threshold, which will be different between experimental procedures and different probe sets. So, before automation, RS-FISH must be used manually on a few images for each signal type you wish to identify.

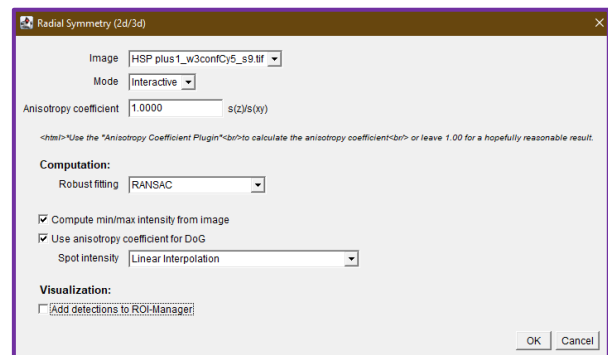
A step by step explanation is provided here, but to gain a full understanding of the process, it is recommended to read the [documentation provided with the plugin](#). Make sure to follow the link provided in the introduction to install RS-FISH before continuing.

### Threshold Value Identification:

1) Open an mRNA channel image and activate the plugin (Plugins → RS-FISH → RS-FISH).

2) A window will open. Under 'Mode', choose 'Interactive. Under 'Robust fitting', choose RANSAC. Mark both tick boxes and finally, under 'Spot intensity', choose 'Linear Interpolation' (it should look like it does in the image). Click ok.

2



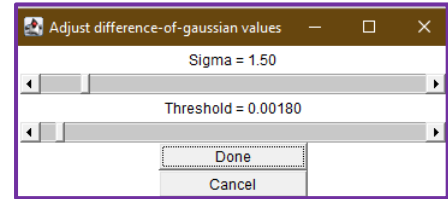
3) Several windows will pop up. Move the additional image window side by side with the original image.

4) If you haven't done so before, change the image's brightness and contrast so you can clearly see signals. Drag and move the square that appeared in your image, so it'll mark an area with signals in it. You can also manipulate its size and shape to your liking, though it shouldn't be larger than a couple of yeast cells. Zoom in to it on both images.

5) Now, when you move across different slices, you can see signals are marked by a red circle. For each spot, across all planes, only the most intense is marked.

- 6) In the 'Adjust difference-of-gaussian values' window (pictured), you can change the Threshold value. Change it several times and test to see the best value, which allows you to

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capture as many signals as possible, without marking noise. **Always prefer to miss some signals (a false negative) than to mark noise as signal (false positive).**

- 7) Once an appropriate value is found, you may check it by repeating the process on a second and third image with the same signal source.
- 8) The threshold value can be used on all images with the same signal source (a specific probe or other marker), who were subjected to the same capturing method (same channels, same microscope, same exposure time, etc.).

## 9. smFISH spots identification using RS-FISH:

Once you have an appropriate threshold for your FISH signals, you can use the automation script to collect data regarding the locations of all spots in your images:

### Script Steps:

- 1) The user is asked for the location of the smFISH channel images directory.
- 2) The user is asked for the spots identification threshold value.
- 3) Once these are inputted, the script performs the following steps on each image:
  - I. Opens smFISH image.
  - II. Runs RS-FISH, using the provided spots threshold.
  - III. Sorts results by 'z' value (the z plane where it was found).
  - IV. Saves results using current image name.

Result tables will be saved as csv files, under the same directory as the smFISH channel images, in a new directory called 'smFISH Spots'.

The output table for each image will include data on each point (from left to right): x, y, z coordinates (width, height and z-plane, respectively), time (only relevant for time lapse images), channel (not relevant with single channel images) and intensity.

x	y	z	t	c	intensity
1519.123	65.0544	1.1473	1	1	252.9637
1203.276	48.6669	1.1954	1	1	120.9883
1588.631	85.913	1.2808	1	1	357.8814
211.1326	1167.16	1.3055	1	1	246.1136
582.0259	364.5312	1.4322	1	1	221.7589



## 10. Colocalization of smFISH signals and organelle:

Once the organelle is well defined in mask files and smFISH spots data is obtained via RS-FISH, colocalization can be examined. This script is the most complex one, so a general description of its steps is provided, instead of a step-by-step account.

First, this script will prompt the user for the locations of the cells mask images, spots csv files created by RS-FISH (if you wish to identify spots using another method, provide a csv file with the same structure as the RS-FISH output as presented above).

Next the script will ask the user whether the organelle was sub-segmented (as described in section 5 of this guide). If it was, choose 'Yes'. If it wasn't, choose 'No'. According to the user's answer, they will be prompted to provide locations of organelle and proximity marker masks (only in case organelle sub-segmentation was performed) and a minimum value for the organelle coverage (for nucleus, we used 0.05, for 5% cell coverage). Make sure you input the binary mask files' directories, and not the statistical map files' locations.

The user will be asked where they want to save the results tables and lastly, for the value of the smFISH signal diameter, in pixels. We recommend using 1, as this will only look for signals 1 pixel away from the spot. If you wish to choose a different value, you can examine several images closely, by zooming into several isolated signals and count the number of pixels making their diameter. Colocalization will be measured according to the diameter chosen. A large diameter may mark signals far from the organelle as colocalized, and a small one will result in less signals identified as associated with the organelle. Take that into consideration when choosing this value. Make sure to use the same signal diameter when examining the same signal source (the same FISH probe set and target, for example).

After the user inputs the values and folders, the script goes over each image, each plane within that image, each cell and each mRNA. Each mRNA is matched with its specific cell, as well as given a colocalization assignment to the organelle. Colocalization is defined by having **more than 0** organelle signal within the diameter provided by the user. In case both sub-section signals are present, the one with a greater total signal (i.e., more organelle signal pixels) is chosen. In case of a tie between the signals, the check will be repeated sequentially, with bigger diameters (starting from 1), until the tie is broken. The check is done by creating a circle with the provided

radius around the smFISH spot's maxima and measuring the amount of organelle signal inside that circle (using basic ImageJ commands). Note that since RS-FISH uses data from multiple slices to identify a spot's location, a spot's 'z' value can be smaller than 0 or bigger than the actual number of cross sections in the image (meaning, it's located at a theoretical point, beyond the limits of the image). In these cases, the spot will be ignored, since there is no organelle data for that location. The number of spots of this nature, if any exist, is very small. In addition, the z plane chosen for colocalization measurement will be the nearest one to each spot.

The script filters out cells according to a few features: edge cells (containing pixels found in the 2% nearest to the edges), cells with less than the input cellular area of proximity marker signal, per plane.

For each image, a CSV file is created in the folder the user inputted. The results will be saved in the following format: Cell #, total number of mRNAs in cell, number of mRNAs of each localization type (organelle near/far, non-organelle), a string with coordinates for each spot within that cell (X, Y values, plane, spot intensity, localization determined) and the organelle coverage value for each z-plane in the image.

Cell #	Total mRNA per Cell	Total Colocalized With Organelle Proximal	Total Colocalized With Organelle Distal	Total Not Colocalized with Organelle	Spots Coordinates Intensity and Colocalization (Far Near or Not Colocalized)	Organelle Signal Size Cross Section 1	Organelle Signal Size Cross Section n
12	3	0	1	2	[[349.8452,131.7305,11,155.3453,organelle_distal], [312.4651,136.6522,13,161.7325,nc], [329.2508,163.6214,13,147.0048,nc],]	24.58628	37.65001
31	1	1	0	0	[[1411.1692,295.842,5,174.1844,organelle_proximal],]	56.23467 ...	43.35384
34	1	0	0	1	[[1376.327,323.9262,13,202.1867,nc],]	0.11074	1.35897

These files can be analyzed by any means you wish, but we recommend the use of our analysis toolset. A user guide for it can be found in the next section.

### Crucial notes:

The script must run on folders containing only the images described above and no other file or folder. Using the previous scripts in the guide to create these images will make sure all files are saved and ordered appropriately. Lastly, the script cannot run in batch mode. Meaning, it must open each image it uses and should not be interrupted while it works. **It is recommended you do not use the computer while it is running** (it's quick, but if you have many images, try to run it on

2-3 of them to evaluate how long you need to leave your computer. ~50 images shouldn't take more than 15 minutes).

**Unresolved Bugs or issues:**

At times, an empty results table window remains open after the script is done. It can be ignored and closed.

The log will be filled with lines only containing '1'. This is an output of the file deletion function and can be ignored.

## 11. Colocalization data analysis tool:

To allow easy analysis of the EASI-ORC output files, we developed and provided several tools written in python, which can be used easily locally or online, via Jupyter Notebook or Google Colab, respectively (the local version requires Python installation, while the online one doesn't). The notebooks also include directions for each step, before each code cell. There's no need in coding to use the analysis tool. Only pressing the 'run' button of each function you wish to activate (filtering, graphing and statistical analysis). However, if used locally, you will need to install Python and several packages.

Here, we explain what the analysis tools provide. **Specific instructions for each section can be found in the analysis tool notebook itself.**

The analysis tool can be divided into four sections:

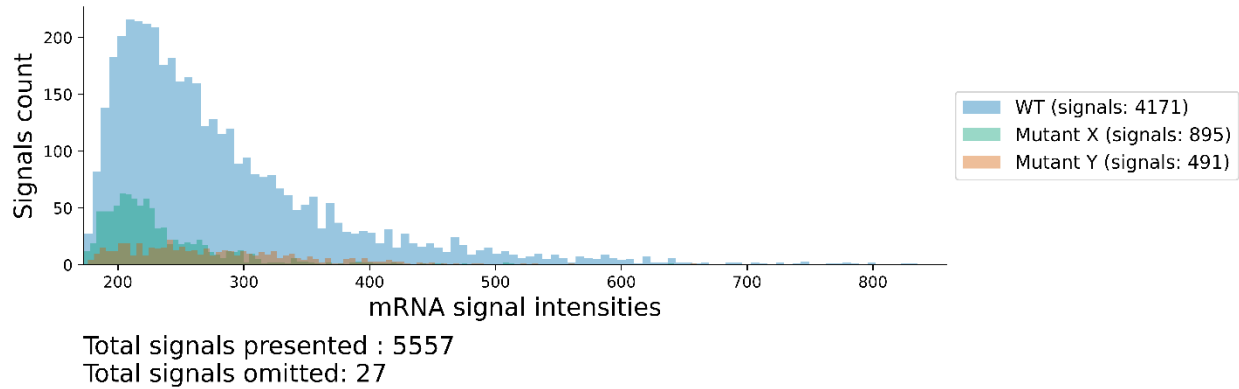
- 1) Table consolidation.
- 2) Data Filtering.
- 3) Data Visualization.
- 4) Statistical Analysis.

Section 1 asks the user how many different treatments/strains are present in their assay. In the online version, it will prompt the user to select the files for each treatment and name each one (This name will appear in results tables and graphs). In the local version, the user is asked for the folder containing the results, identification strings of each sample (must be specific and case sensitive) and the name the user wishes to give each sample for result tables and graphs. It will then create a consolidated table for each treatment, which can be used in the next sections.

Section 2 provides several filtering steps, presented visually and updated as you input new filtering values. The filters must be performed in the order in which they appear (if you change one, you must also change the next ones). Each filter has minimum and maximum values. First, a filter of smFISH spots by spot intensity. Second, a filter of cross sections by cellular organelle signal coverage (no organelle signal will lead to non-colocalized designation to all FISH spots, while too much signal will likely result in bias to designate all signals as localized). Lastly, you may filter the data by the number of FISH signals per cell. This section also creates a new table for each treatment of the data with the filters applied to it (a filtered table). Filter values can be

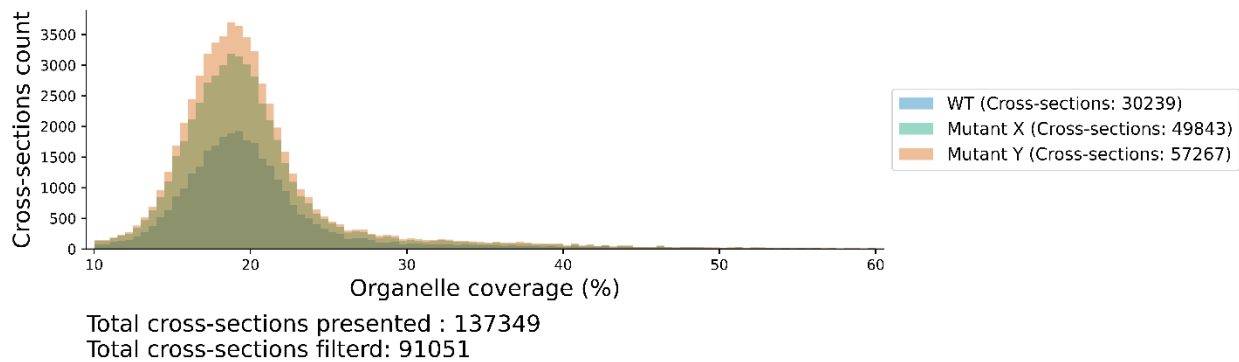
inputted directly (double clicking the number and changing it) or using the sliders above the graph.

### intensity per mRNA signal



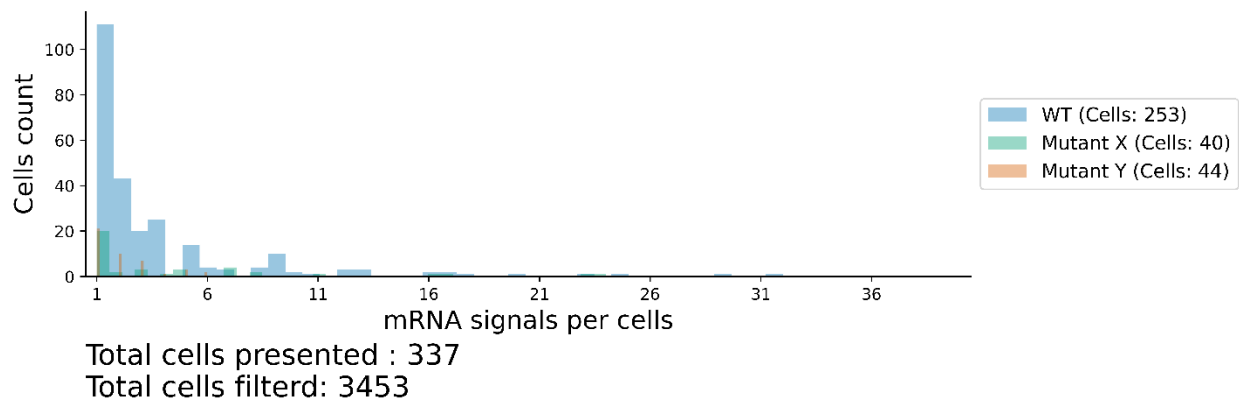
### Filter 1 – Intensity per mRNA signal

#### Organelle coverage of cell per cross-section



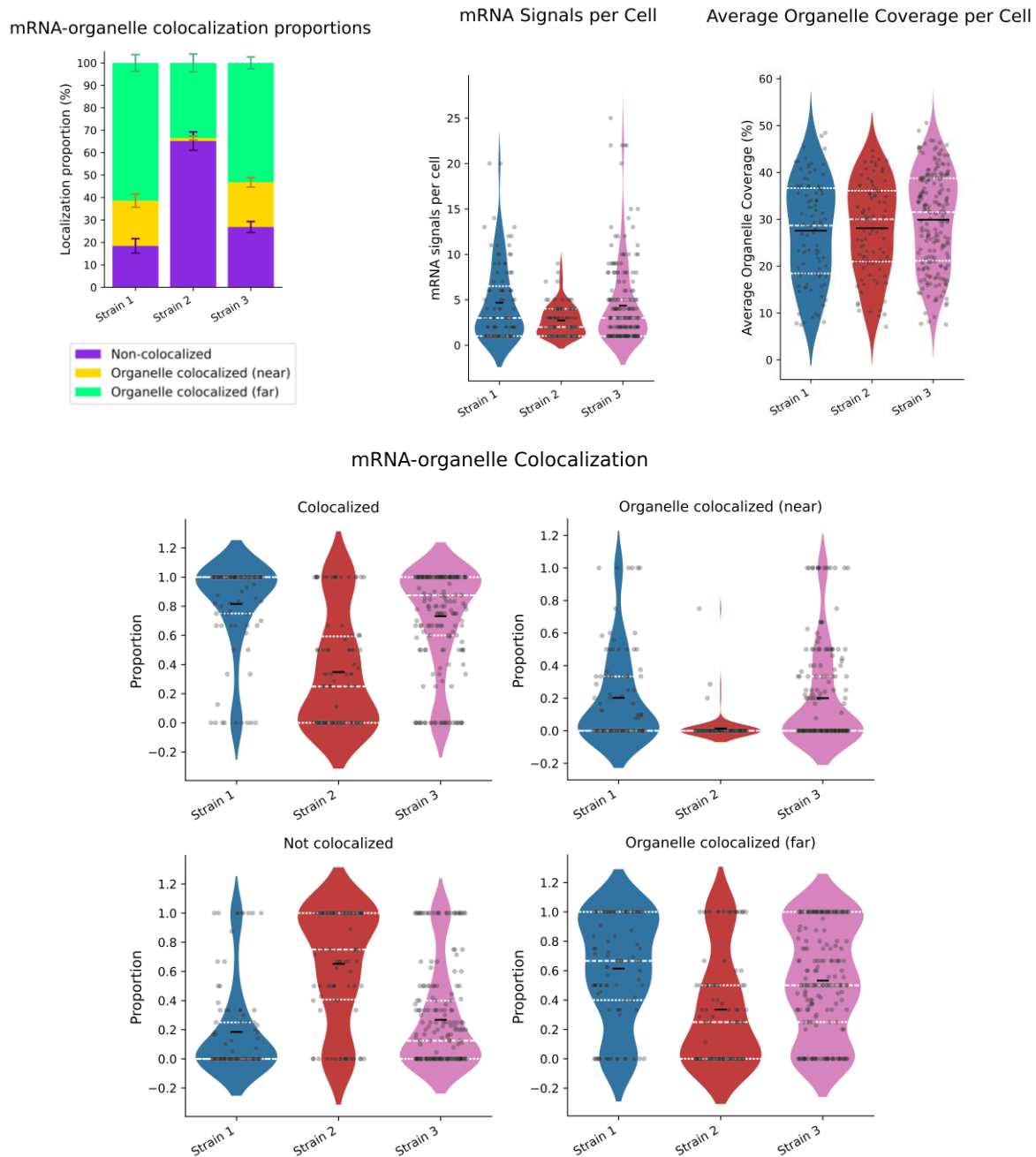
### Filter 2 – Organelle coverage of cell per cross-section

#### Number of mRNA signals per cell



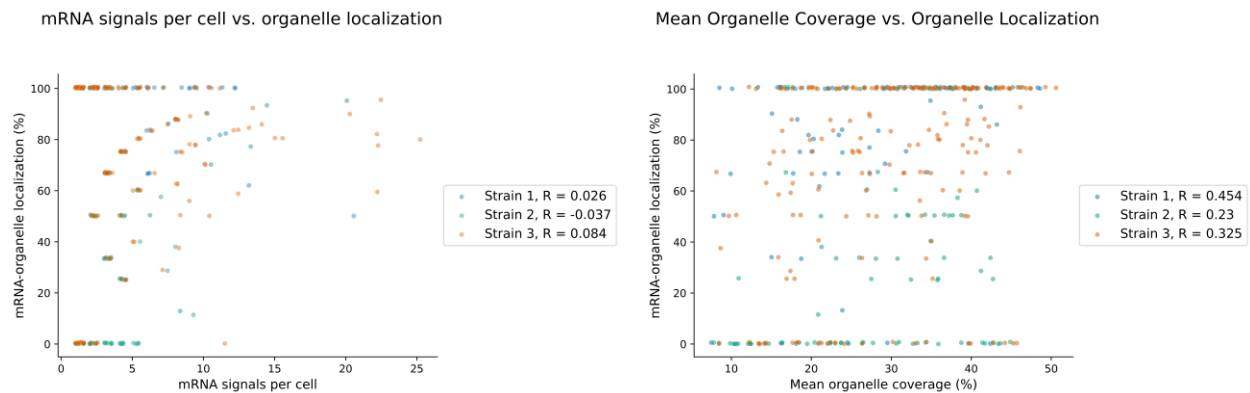
### Filter 3 – Number of mRNA signals per cell

Section 3 provides several graphs for colocalization analysis: A proportion graph presenting organelle localization (or the proximal and distal organelle sub-segments, if sub-segmentation was performed) percentages, violin graphs of each colocalization sub-population (proximal, distal, non-colocalized and total colocalized proportions), and violin graphs presenting the number of mRNA signals per cell, per treatment and average organelle coverage (calculated only from the z planes remaining after filtering for organelle coverage).



**Graphs output by the analysis code.**

In addition, two scatter plots are produced to describe the correlation between mRNA-organelle colocalization proportion with either the number of mRNA signals per cell or the average organelle cellular coverage (the average is calculated between all z planes that were not filtered out).



### mRNA-organelle Colocalization correlation plots

Finally, section 4 provides a table with statistical tests performed for and between each treatment. Each treatment's data set is compared via Student's t-test to every other treatment, to provide statistical significance of differences between each treatment. All results are collected in a statistical table, saves as a csv file (example table below).

Every graph and table produced by the analysis tool is available to save individually and to download together, in a zip file.

Example of Statistical Summary Table

Feature	Strain 1	Strain 2	Strain 3
Number of cells before filtering	1164	690	785
Number of cells after filtering	179	90	99
Percentage of positive cells	15.378007	13.043478	12.611465
mean proportion of non-organelle localization	0.3424406	0.650754	0.3331566
standard deviation proportion of non-organelle localization	0.3311617	0.3849421	0.303338
standard error proportion of non-organelle localization	0.0248216	0.0408038	0.0306418
independent t test with Strain 1 (non-organelle localization) p-Value	1	1.29E-09	0.8140946
independent t test with Strain 2 (non-organelle localization) p-Value	1.29E-09	1	3.70E-09
independent t test with Strain 3 (non-organelle localization) p-Value	0.8140946	3.70E-09	1
mean proportion of proximal organelle localization	0.109664	0.0137302	0.1941591
standard deviation proportion of proximal organelle localization	0.1912716	0.0860985	0.2813304
standard error proportion of proximal organelle localization	0.0143364	0.0091264	0.0284187
independent t test with Strain 1 (proximal) p-Value	1	4.25E-08	0.0088044
independent t test with Strain 2 (proximal) p-Value	4.25E-08	1	1.80E-08
independent t test with Strain 3 (proximal) p-Value	0.0088044	1.80E-08	1
mean proportion of distal organelle localization	0.5478953	0.3355159	0.4726843
standard deviation proportion of distal organelle localization	0.3397583	0.3771238	0.3316722
standard error proportion of distal organelle localization	0.025466	0.039975	0.033504
independent t test with Strain 1 (distal) p-Value	1	1.40E-05	0.075378
independent t test with Strain 2 (distal) p-Value	1.40E-05	1	0.0092901
independent t test with Strain 3 (distal) p-Value	0.075378	0.0092901	1
mean number of mRNA per cell	9.7988827	2.7444444	10.808081
median number of mRNA per cell	6	2	6
standard error number of mRNA per cell	0.7717869	0.189329	1.2844853
independent t test with Strain 1 (Total mRNA per Cell) p-Value	1	4.06E-16	0.5015701
independent t test with Strain 2 (Total mRNA per Cell) p-Value	4.06E-16	1	1.15E-08
independent t test with Strain 3 (Total mRNA per Cell) p-Value	0.5015701	1.15E-08	1
mean of organelle coverages	26.090056	28.06861	25.65967
standard error of organelle coverages	0.7614677	1.0505075	0.89201
independent t test with Strain 1 (filtered organelle coverages mean) p-Value	1	0.1312486	0.7245669
independent t test with Strain 2 (filtered organelle coverages mean) p-Value	0.1312486	1	0.0804421
independent t test with Strain 3 (filtered organelle coverages mean) p-Value	0.7245669	0.0804421	1
Pearson correlation mean organelle coverages vs organelle localizations	0.3475328	0.2341923	0.4330672
Correlation p value mean organelle coverages vs organelle localizations	1.87E-06	0.0263049	7.56E-06
Pearson correlation #mRNA signals in cell vs organelle localization	0.1075409	-0.036583	0.0379133
Correlation p value #mRNA signals in cell vs organelle localization	0.1518925	0.7321083	0.7094643