README clampFISH 2.0 data analysis

This document describes the steps needed to generate all plots and images for this paper.

# **System requirements & installation**

## Software for control of microscope

NIS-Elements v5.11.01 was used on a PC running Windows 10.

NIS-Elements Viewer may also be used to export TIFF files from .nd2 format. It can be [freely downloaded here](https://www.microscope.healthcare.nikon.com/products/software/nis-elements/viewer).

## Software for all other data analysis

All other software, listed below, was run on macOS (Mojave 10.14.6).

| Software package | language | version tested | Download location |
| --- | --- | --- | --- |
| MATLAB | MATLAB | R2021a (64-bit, maci64) | [www.mathworks.com](http://www.mathworks.com) |
| MATLAB Toolboxes | MATLAB |  | [www.mathworks.com](http://www.mathworks.com) (see below list) |
| rajlabimagetools | MATLAB |  | <https://github.com/arjunrajlaboratory/rajlabimagetools> |
| data processing and display scripts\* | MATLAB |  | paper/scripts folder within <https://www.dropbox.com/sh/q51kmcphoyi9yi3/AAB4g1a6ODDHaphsvbmBJAy-a?dl=0> |
| bfmatlab | MATLAB | 6.5.1 | <https://www.openmicroscopy.org/bio-formats/downloads/> |
| cellpose | python | Downloaded 16-Mar-2021 | <https://cellpose.readthedocs.io/en/latest/> |
| anaconda | python | Downloaded 19-Mar-2021 | <https://www.anaconda.com/products/individual> |
| python3 | python | 3.7.10 | from anaconda distribution |

\* data processing scripts folder includes the violinplot function:

Bechtold, Bastian, 2016. Violin Plots for Matlab, Github Project

https://github.com/bastibe/Violinplot-Matlab, DOI: 10.5281/zenodo.4559847

MATLAB Toolboxes

* Image Processing Toolbox
* Statistics and Machine Learning Toolbox
* Computer Vision Toolbox
* Lidar Toolbox
* Navigation Toolbox
* Robotics System Toolbox
* ROS Toolbox
* UAV Toolbox
* Deep Learning Toolbox
* Bioinformatics Toolbox

# Demo and Instructions for Use

All image data processing involves the following 3 steps, each

1. **raw data processing**

*relevant scripts in the rawDataProcessingScripts folder. Outputs can be found in separate directories within the ‘rawData’ folder.*

* 1. converting .nd2 files into TIFF images

*For some experiments tiles were directly exported using NIS-Elements. In others, it was done using the appropriate MATLAB script in the rawDataProcessing folder. Where applicable, the MATLAB scripts output stitched and registered TIFF files. Both the .nd2 files and the output TIFF files are located under the directory rawData.*

* 1. where applicable, images were segmented with cellpose

1. **extraction**

*Relevant scripts in the extractionScripts folder. Outputs can be found in separate directories within the ‘paper/extractedData’ folder.*

* 1. [where applicable] images were manually segmented using rajlabimagetools
  2. automatic spot detection using rajlabimagetools or dentist2
  3. [where applicable] manual cell-specific intensity threshold selection for conventional single-molecule RNA FISH spots
  4. [where applicable] assigning spots from dentist2 to segmented cells
  5. extracting spot data from data0XX.mat files (where XX is a number)
  6. selecting a clampFISH 2.0 spot intensity threshold
  7. writing spot data table (Tspots) and cell data table (Tcell) to ‘extractedData’ folder

1. **plot** **OR** **show image**

*Relevant scripts in the plotScripts folder or showImagesScripts folder. Outputs can be found in separate directories within the ‘paper/plots’ or ‘paper/exampleImages’ folders.*

* 1. Plot: taking data from extractedData folder, and generating a plot (.eps format)
  2. Show image: cropping, contrasting, and merging (with DAPI) TIFF files into RGB format.

The scripts used in these steps read an experiment-specific table of conditions (ending in Tcond.xlsx) in the ‘paper/experiments’ folder, which references the ND2 file names. The scripts may also read tables related to multiple imaging cycles (ending in Tscan.xlsx) and tables related to imaging details (ending in ImagingDetails.xlsx).

The experiments and their associated figures and script names are listed in **ExperimentList.xlsx** in the ‘paper’ folder.

* To re-run a full analysis from the raw .nd2 files, the scripts scripts should be run in the order above (rawDataProcessingScripts → extractionScripts → plotScripts OR showImagesScripts). In this case, you’ll need to download the relevant ND2 files from the ‘rawData’ folder.
* To re-run an analysis starting from the TIFF files, you can skip the initial ‘raw data processing’ step. In this case, you’ll need to download the relevant TIFF files from the ‘rawData’ folder.
* To re-run an analysis from after data extraction, you can skip the ‘raw data processing’ and ‘extraction’ steps and just run the appropriate MATLAB script from ‘plotScripts’ or ‘showImagesScripts’. In this case, you won’t need any data from the ‘rawData’ folder, and instead will just need the data from the ‘paper/extractedData’ folder.

File/folder paths in scripts and in the experiment-level tables (files ending in Tcond.xlsx) may require editing of file and folder path locations to match local directories.

All expected outputs for the scripts are placed in their appropriate folder locations, indicated above. Where processing time takes many hours, this has generally been noted in a comment in the applicable script.