DrSTAR User guide

System requirements:

1. Windows or Mac
2. MATLAB 2020b with:
    a. Signal Processing Toolbox
    b. Image Processing Toolbox
    c. Computer Vision Toolbox
    d. Parallel Computing Toolbox

    NOTE: Tested on MATLAB 2021b as well
3. Free disk space - at least 1.5x the size of raw dataset

    NOTE: For 8 cells (“Full Optosplit data” Test dataset - 63 GB) approximately 63 GB of free space are needed. For convince of debugging and package testing smaller data set is provided as well (”Truncated Optosplit data” - 1GB).

Installation:

1. Download the program from the GitHub:

    https://github.com/Mattheyses-Lab/Nawara_et_al._BJ_2022.git

2. Make sure that the folder containing the DrSTAR package is set as a working directory or add the package folder to MATLAB search path
3. Open DrSTAR.m in MATLAB 2020b

Data supported:

NOTE: DrSTAR supports 488 and 647 nm wavelengths to align with the iRFP713-EGFP STAR probe. The program can be upgraded to support other wavelengths.

1. Image splitter (ex. OptoSplit III)
2. Dual-camera system

Test dataset:

To be processed dataset (FBS 06.02.20) and dataset post-processing with DrSTAR (FBS 06.02.20 – Processed) can be found under the following link in the “Full Optosplit data” folder:

https://bit.ly/DrSTAR_TestDataset

Under the same link we also provide a smaller version of the test dataset (”Truncated Optosplit data” folder, only two cells with truncated time lapse) that is helpful for program debugging and testing. DrSTAR supports data input from a dual-camera systems and a mock data set is under the same link (“Mock dual-camera system data” folder).

Required inputs for DrSTAR (graphical abstract included here):

1. **y** – Distance between the laser smear and the objective on the wall (**Fig. S4**).
    a. Test dataset:
        i. y = 55
2. **Microscope system setup** – specifies whether image splitter or dual camera system was used.
    a. Test dataset:
        i. OS
3. **Live cell data**
    a. Test dataset:
        i. OS_CLC-STAR_FBS.002.nd2
        ii. OS_CLC-STAR_FBS.003.nd2
        iii. OS_CLC-STAR_FBS.101.nd2
        iv. OS_CLC-STAR_FBS.101.nd2
        v. OS_CLC-STAR_FBS.102.nd2
        vi. OS_CLC-STAR_FBS.103.nd2
        vii. OS_CLC-STAR_FBS.201.nd2
        viii. OS_CLC-STAR_FBS.202.nd2
        ix. OS_CLC-STAR_FBS.203.nd2
4. **Flat field correction images for each wavelength**
    a. Test dataset:
        i. OS_FF488.003.nd2 - 488 nm flat-filed image

ii. OS_FF647.003.nd2 - 647 nm flat-filed image

5. **NanoGrid image** – for OptoSplit III calibration
    a. Test dataset:
        i. OS_Cali.005.nd2

How to run DrSTAR:

1. Open DrSTAR in MATLAB 2020b
2. Make sure the folder contain package is added to MATLAB search path or set the folder as a working directory
3. Press run

DrSTAR step-by-step (Fig. S2):

1. Organize data

    NOTE: DrSTAR requires data files to be organized and labeled in a particular way (**Fig. S1**). When using an Optosplit based system - all data has to have a prefix “OS_”, which indicates that images were taken using an image splitter which directs two wavelength channels to different regions of a camera, such as the OptosplitIII (Cairn Research), then: 1) The NanoGrid image stack should be followed by “Cali.” and the number of the picture (i.e. “OS_Cali.001.nd2”, “.nd2” indicates the file type and it is characteristic for Nikon Elements version 5.02). 2) The flat field corrections files should be followed by “FF”, the wavelength used for imaging “488” or “647” and the number of the picture (i.e., “OS_FF488.001.nd2”, “OS_FF647.003.nd2”). 3) The live-cell data should be followed by the name of the imaged protein followed by the indication that it is STAR tagged, experimental condition, and cell number (i.e., “OS_CLCa-STAR_Ctrl.001.nd2”).

    NOTE: When using a dual camera system - all data has to have a prefix “DC_”, then: 1) The NanoGrid image stack should be followed by wavelength and “Cali.” and the number of the picture (i.e. “DC_488_Cali.005.nd2”, “DC_647_Cali.005.nd2”). 2) The flat field corrections files should be followed by “FF”, the wavelength used for

imaging and the number of the picture (i.e., “DC_FF488.001.nd2”, “DC_FF647.001.nd2”). 3) The live-cell data should be followed by the name of the imaged protein followed by the indication that it is STAR tagged, experimental condition, wavelength, and cell number (i.e., “DC_CLCa-STAR_488_Ctrl.001.nd2”, “DC_CLCa-STAR_647_Ctrl.001.nd2”).

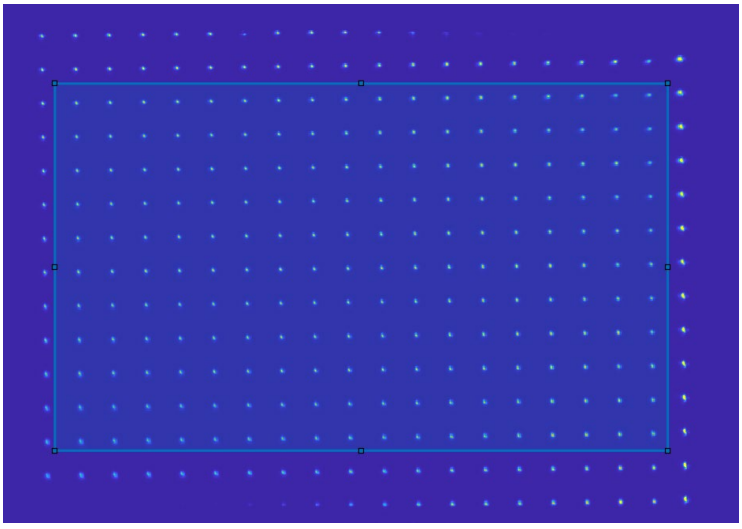
2. Press run
3. Choose the directory containing the data set (ex. FBS 06.02.20)
4. Provide y (ex. 55)

   NOTE: Depending on your microscope set up the x value – the distance between objective and the wall – will have to be adjusted in gamma_calculator.m

   NOTE: Depending on your experimental setup wavelengths used for the imaging and glass, prism and cell refraction indexes must be adjusted in gamma_calculator.m
5. Specify whether OptoSplit or dual camera system was used to acquire the data (ex. OS)

   NOTE: Make sure that data is labeled in appropriately
6. Once the NanoGird image appears, draw rectangle specifying the 488 nm emission area (ex. ROI around top grid holes)

   NOTE: Leave the most outer set of grid holes outside the ROI (see below)



NOTE: Do not change the size of the ROI

NOTE: If redrawing is necessary press Redraw

7. Drag the rectangle to the corresponding 647 nm area (ex. Bottom grid holes)

   NOTE: Do not change the size of the ROI

8. Press OK on the small popup window
9. Align the 488 and 647 grid holes

   NOTE: First align X then Y, if X need to be readjusted the Y will need to be reapplied

10. Press OK in the MATLAB App
11. Press OK on the small popup window

    NOTE: There will be a wait time before the next step is required

12. Once cell picture appears draw ROI outside of the cell area (background ROI)

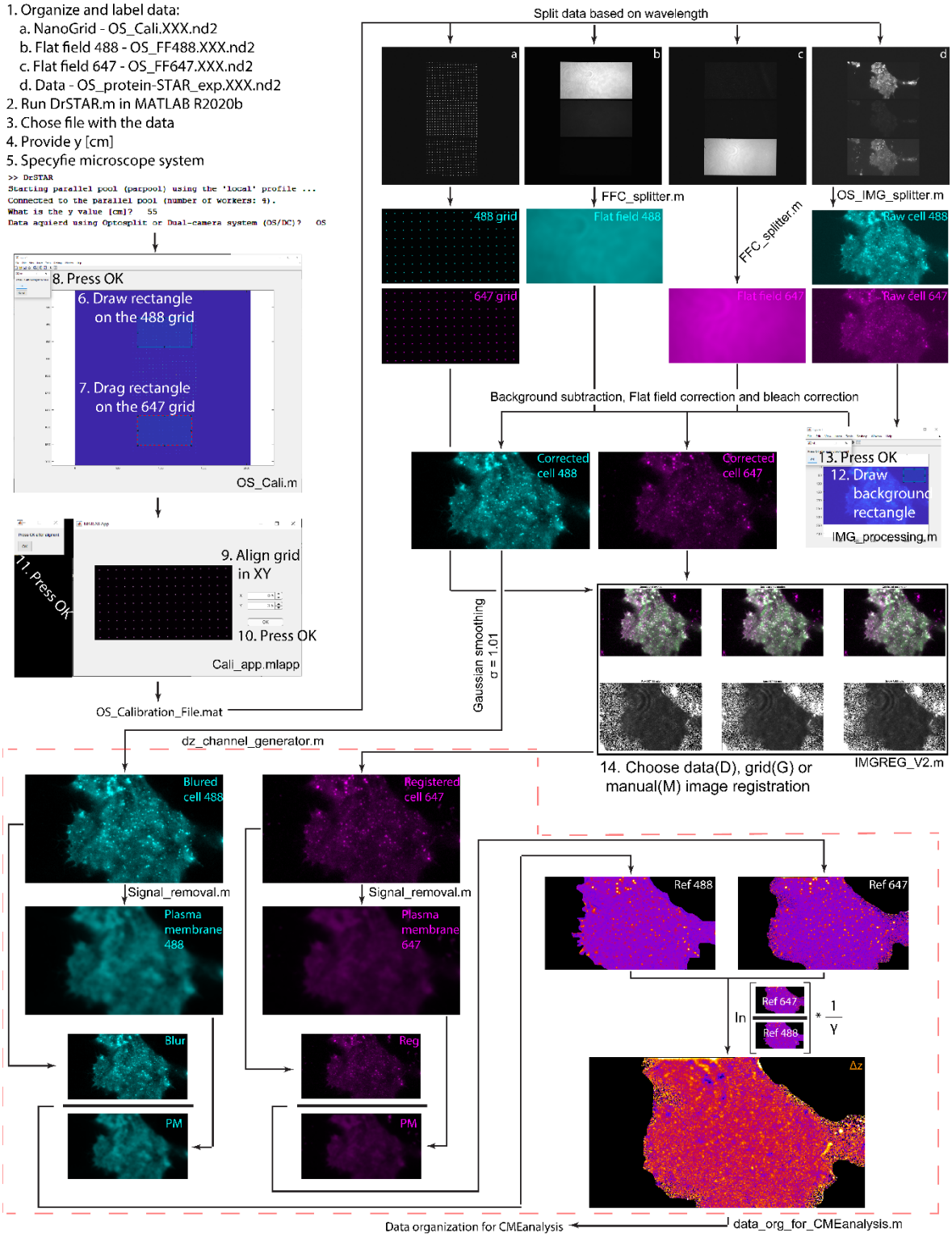
    NOTE: Step 11 and 12 has to be repeated for every cell separately

13. Press OK on the small popup window
14. Once the Registration figure will appear, examine the registration, and select best registration results by typing in command line: D (data-based registration) or G (grid-based registration). (ex. G)

    NOTE: Do not close the registration figure, if you want it to be saved

    NOTE: If both data-based and grid-based registration are not to your satisfaction type M in command line to manually select the (*x, y*) coordinates one at the time, then close the window, examine the result, and type Y in command line to continue and confirm registration method or N to come back to data-based and grid-based registration.

    NOTE: This step has to be repeated for every cell, after that no further supervision is required.



Step-by-step explanation of the DrSTAR algorithm. First data must be organized as described in **Fig. S1**. Then user can run DrSTAR, select the folder that contains the raw data, and provide the y value (**Fig. S4**). If available, parallel computing will initiate

automatically. Next, user specifies microscope system used and draws ROI indicating the 488 nm emission and then drags the rectangle to the 647 nm emission part of the camera chip (do not change the size of ROI). This will result in raw data splitting based on emission. Next, user must pick the background ROI for each cell followed by choosing the registration algorithm (**Fig. S6**) for each cell. Form here calculation of local background (**Fig. S5**), and Δz channel, and data organization for CME_analysis proceeds automatically.

Data organization post DrSTAR processing:

Along the raw data three new folders are created:

1. Corrections – contains the files used between cells, such as the split NanoGrid images (ex. Grid_488_raw.tif) and flat-fields images (ex. OS_FF488), the y value (ex. y.mat), data_type note (ex. data_type.mat), calibration file (ex. Calibration_File.mat), and text files noting completion of steps (ex. 647_Registration_done.txt) – required for program to finish from last completed step if re-running is needed.
2. Data_analysis – contains the half products of correction process for each cell, such as, raw split data (ex. OS_CLC-STAR_FBS.002_488_Raw.tif), background subtracted, flat field corrected and bleach corrected data (ex. OS_CLC-STAR_FBS.002_488_Cor.tif), signal mask (ex. OS_CLC-STAR_FBS.002_488_Signal_mask.tif), cell mask (ex. OS_CLC-STAR_FBS.002_cell_mask.tif), calculated plasma membrane image stack (ex. OS_CLC-STAR_FBS.002_488_PM.tif), the registration figure (ex. OS_CLC-STAR_FBS.002_reg_fig.png), the transformation from (ex. OS_CLC-STAR_FBS.002_tform.mat)
3. For_CMEanalysis – contains data sorted and ready for direct input into CME_analysis for each cell, such as the corrected and blurred 488 nm signal (Ch1 - ex. OS_CLC-STAR_FBS.002_488_Cor_Blur.tif), corrected and registered 647 nm signal (Ch2 - ex. OS_CLC-STAR_FBS.002_647_Cor_Reg.tif) and Δz (Ch3 - OS_CLC-STAR_FBS.002_dz_channel.tif)