Packaged RoSEO3D

# Download the example data

https://osf.io/b7m8r/?view\_only=fe63203e28524a3d960f1d84345a7c5a

# Analyze the data

## 1. prepare the running:

a**) using Thunderstorm in ImageJ to estimate the 2D locations of SMs**. This is a rough estimation and the estimation will be used to register the x-y channel and calculate the background.

\* a name follows the format of data#\_xycho\_

Graphical user interface, application, Word

Description automatically generatedGraphical user interface, application, table

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b) **modify the excel file for your data’s parameter.**

\20230208 SLB\processed data RoSEO\RoSEO\_dataSheet\20230208\_RoIsheet\_data26\_31\_FoV1\_demo.xlxs

* change the file location as indicated by ###
* Change all the other parameters that are relevant (e.g., data set, FoV, RoSEO estimation parameter)

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## 2. Run the estimation

**input excel to Matlab and run the code**

* main\_RoSEO3D\_preprocessing/run\_main\_RoSEO3D.
* modify the name and location of the excel name

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* Ideally, you should be able to run packaged code that includes the registration, image cropping, offset cropping, background estimation, RoSE3D estimation.
* The code will automatically generate a new folder to save the estimation results and intermediate visualization figures. You can define the name of the new folder inside excel.
* Notably, as different people have different habits to name the data file, sometimes you might need to go into each subfile to modify how the code reads the file name.

# Visualize the estimation

## 1. Prepare your visualization

* 3D SLB vidualization\_demo\SLB data\ data\_overview.m
* Motivation: it happens that we have data that separate into different stacks, or we have data from different samples.
* Data\_overview.m lists the information of different dataset into cases to allow us to modify/add/delete cases easily

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## 2. 6D visualization

* 3D SLB vidualization\_demo\ SMLM\_stackes\_combine.m
* Please modify the code to fit into your visualization goal
* The current code is designed to visualize the spherical lipid membranes: 1) x-y view of the phi angle at different axial positions, 2) x-y, x-z view of the theta angle, 3) x-y-z view of the theta angle
* The results will be saved into defined folder

Graphical user interface, website

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## 3. Reconstruction check

* 3D SLB vidualization\_demo\ check\_reconstruction.m
* It will generate hundreds frames for your to check if the reconstruction match with the raw image well.

A picture containing background pattern

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