User guide for calculating MPCC

Package contents

There are three codes in the package: MPCC.m, Uni_3D.m and pixelate_image.m. The package also includes a sample data set for calculating MPCC, named 'Synthetic_Data.mat'. We have also included a folder titled 'Preanalyzed Data'. The folder contains an example of calculated MPCC between two image matrices, 'red_mat' and 'green_mat'.

Details of included codes

These three codes necessary for calculating MPCC should be stored in the same folder. We recommend using the latest version of MATLAB® (R2017b) for running the codes.

1. **MPCC.m:** This code calculates the MPCC between 2 images (**R** and **G**, as described in manuscript). The user needs to provide **R**, **G**, $\mathbf{U^R}$ and $\mathbf{U^G}$. The code also provides the scatter plot of $\hat{\Delta}_{ij}^{R}$ vs. $\hat{\Delta}_{ij}^{G}$ for visualization purposes.
2. **Uni_3D.m**: This code generates 3D random distributions of red and green molecules in a spherocylinder of chosen dimensions. The user needs to input number of red and green molecules in the distribution, localization error for red and green molecules and the length and radius of the spherocylinder.

   In addition the user can choose to generate 2D localization probability density heat maps of a chosen pixel size for the simulated localizations of red and green molecules.
3. **pixelate_image.m**: This code generates 2D localization probability density heat maps of a chosen pixel size for localizations of red and green molecules. The user needs to input the *x* and *y* localizations of red and green molecules, length and radius of the cell as well as the choice of pixel size.

Calculating MPCC for a simulated data set

The package includes a sample dataset, 'Synthetic_Data.mat'. There are 4 image matrices in this dataset: red_mat (**R**), green_mat (**G**), uni_red_mat ($\mathbf{U^R}$) and uni_red_mat ($\mathbf{U^G}$). For calculating MPCC of red_mat and green_mat, 'Synthetic_Data.mat' must be loaded into the workspace. The user needs to hit run for 'MPCC.m'. The prompt window for the four input parameters will appear. The user needs to input the name of the four image matrices i.e **R**, **G**, $\mathbf{U^R}$ and $\mathbf{U^G}$. Currently, the default names are set according to the 'Synthetic_Data.mat'.

Once the code is executed, the calculated MPCC value for red_mat and green_mat as well as the scatter plot of $\hat{\Delta}_{ij}^{R}$ vs. $\hat{\Delta}_{ij}^{G}$ will be displayed.

Calculating MPCC for an experimental data set

For calculating MPCC between two experimental spatial distributions using the provided package of codes, the experimental data should be modified appropriately. The cell length and radius of the imaged cell must be determined prior to analysis. The obtained *x* localizations of red and green molecules must be normalized with respect to the cell length such that 0 represents

the cell center and ± 0.5 represent the locations of the two cell tips axially. Similarly the obtained *y* localizations of red and green molecules must be normalized with respect to the cell width such that 0 represents the cell center and ± 0.5 represent the locations of the two transverse cell tips. In cases where the *x* and *y* localizations are outside the measured cell length and width owing to localization error, the new scaled localizations are greater than a magnitude of 0.5.

These scaled *x* and *y* localizations can be converted into 2D probability density heat maps using 'pixelate_image.m' to give **R** and **G**. The two 2D reference matrices ($\mathbf{U^R}$ and $\mathbf{U^G}$) required for calculating MPCC corresponding to red and green channels can be generated using 'Uni_3D.mat'. The generated **R**, **G**, $\mathbf{U^R}$ and $\mathbf{U^G}$ matrices need to be loaded in the workspace. Execution of 'MPCC.m' will calculate MPCC between **R** and **G**.

Notes:

R, **G**, $\mathbf{U^R}$ and $\mathbf{U^G}$ matrices must have same number of rows and columns.

R, **G**, $\mathbf{U^R}$ and $\mathbf{U^G}$ matrices must have same size of pixels.