DNA-PAINT super-resolution imaging data of surface exposed active sites on particles

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Introductory Information

The shared data consist in a set of single-molecule localization imaging acquisitions of binding sites exposed on the surface of streptavidin-coated polystyrene particles. Data were obtained with DNA Points-Accumulation for Imaging in Nanoscale Topography (DNA-PAINT) procedure by loading streptavidins with biotinylated DNA docking strands and then introducing a dye-conjugated DNA imager strand in solution for single-molecule localization. The reported data were obtained in four different types of experiments and have been organized accordingly in sub-sections:

- **1COLORdata:** two-dimensional DNA-PAINT imaging data for particles and for a control sample obtained with wrong pairing of docking-imager DNA strands;
- **THREE-DIMdata**: three-dimensional DNA-PAINT imaging data for particles;
- **MULTI-COLORdata**: two-dimensional DNA-PAINT imaging data for particles, consisting in three different acquisitions on the same field of view, corresponding to different pairs of docking-imager DNA strands;
- **TITRATIONdata**: two-dimensional DNA-PAINT imaging data for multiple particles samples exposing an increasing amount of docking DNA strands on the surface;
- **Matlab codes**: analysis functions to obtain single particle parameters;

Methodological Information

Data collected using a total internal reflection microscope N-STORM from Nikon. Raw time-lapse records are reported as TIF files. Raw records were analysed with a NIS-element software from Nikon, to identify single-molecule parameters. Results of this analysis are provided as TXT files.

Data specific information

File names, acquisition and analysis parameters are reported below for each specific experiment

1COLORdata

General description:
streptavidin-coated polystyrene particles (320 nm diameter) incubated with biotin-conjugated DNA docking strand in excess (10 µM) at different concentrations; washed and imaged on glass slide in imaging buffer (5 Mm Tris-HCl, 10 mM $MgCl_2$, 1mM EDTA, 0.05% Tween-20, pH = 8) containing complementary DNA imager strand conjugated with Atto647N at 0.1 nM.

Acquisition parameters:
256x256 pixels region of camera, pixel size = 160nm
total internal reflection irradiation
main excitation = 647 nm (50% of max power)
reference excitation = 561 nm (0.5% of max power), 1 frame every 100 of main-channel
camera exposure time = 50ms (20 Hz)
50000 frames in main channel
Max power 647 nm laser = 160 mW
Max power 561 nm laser = 80 mW

Analysis parameters in NIS-elements:
Camera baseline value = 100
Minimum width (nm) = 200
Maximum width (nm) = 700
Max. axial ratio = 1.3
Max. displacement (pix) = 1
minimum intensity threshold main channel = 200
maximum intensity threshold main channel = 20000
minimum intensity threshold reference channel = 400
maximum intensity threshold reference channel = 20000
time-trace length = 1-10 frames

Raw data file name	Analysed data file name	Docking-imager sequences pairing
2017-05-11_1.tif	2017-05-11_1.txt	*Complementary*
2017-05-11_2.tif	2017-05-11_2.txt	*Complementary*
2017-05-11_3.tif	2017-05-11_3.txt	*Non-complementary*

Name of main channel in txt files: '*647*'
Name of reference channel in txt files: '*Bead Drift Correction*'

THREE-DIMdata

General description:
streptavidin-coated polystyrene particles (320 nm diameter) incubated with biotin-conjugated DNA docking strand in excess (10 μM) at different concentrations; washed and imaged on glass slide in imaging buffer (5 Mm Tris-HCl, 10 mM $MgCl_2$, 1mM EDTA, 0.05% Tween-20, pH = 8) containing complementary DNA imager strand conjugated with Atto647N at 0.1 nM.

Acquisition parameters:
256x256 pixels region of camera, pixel size = 160nm
total internal reflection irradiation
cylindrical lens in
main excitation = 647 nm (50% of max power)
reference excitation = 561 nm (1% of max power), 1 frame every 100 of main-channel
camera exposure time = 50ms (20 Hz)
40000 consecutive frames in main channel
Max power 647 nm laser = 160 mW
Max power 561 nm laser = 80 mW

Analysis parameters in NIS-elements:
Camera baseline value = 100
Minimum width (nm) = 200
Maximum width (nm) = 700
Max. axial ratio = 2.5
Max. displacement (pix) = 1
minimum intensity threshold main channel = 250
maximum intensity threshold main channel = 20000
minimum intensity threshold reference channel = 400
maximum intensity threshold reference channel = 20000

z-calibration parameters:
Range = -400 to 400 nm, Step = 1.0 nm, Tolerance = 1.5, Wavelength = 647
Wx0=293.2; zrx=412.6; gx=205.8; Ax=-0.1688; Bx=0.2886; Cx=Dx=0;
Wy0=282.8; zry=345.4; gy=205.8; Ay=-0.2749; By=0.06191; Cy=0.04366; Dy=0;

Raw data file name	Analysed data file name
2017-07-05_0001.tif	2017-07-05_0001.txt

Name of main channel in txt files: '*647*'
Name of reference channel in txt files: '*Bead Drift Correction*'

MULTI-COLORdata

General description:
streptavidin-coated polystyrene particles (320 nm diameter) incubated with biotin-conjugated DNA docking strand; four populations of particles prepared exposing different DNA docking strand sequences: *docking1, docking2, docking3, docking1-2-3*; washed and imaged on glass slide in imaging buffer (5 Mm Tris-HCl, 10 mM $MgCl_2$, 1mM EDTA, 0.05% Tween-20, pH = 8) containing DNA imager strand conjugated with Atto647N at 0.1 nM.
Same field of view was imaged 3 times sequentially flowing in the imager solution and washing

Acquisition parameters:
256x256 pixels region of camera, pixel size = 160nm
total internal reflection irradiation
main excitation = 647 nm (50% of max power)
reference excitation = 561 nm (1% of max power), 1 frame every 100 of main-channel
camera exposure time = 50ms (20 Hz)
20000 consecutive frames in main channel
Max power 647 nm laser = 160 mW
Max power 561 nm laser = 80 mW

Analysis parameters in NIS-elements:
Camera baseline value = 100
Minimum width (nm) = 200
Maximum width (nm) = 700
Max. axial ratio = 1.3
Max. displacement (pix) = 1
minimum intensity threshold main channel = 200
maximum intensity threshold main channel = 20000
minimum intensity threshold reference channel = 400
maximum intensity threshold reference channel = 20000
time-trace length = 1-10 frames

Raw data file name	Analysed data file name	imager DNA strand sequence
2017-06-23_0001.tif	2017-06-23_0001.txt	*Imager 1*
2017-06-23_0002.tif	2017-06-23_0002.txt	*Imager 2*
2017-06-23_0003.tif	2017-06-23_0003.txt	*Imager 3*

Name of main channel in txt files: '*647*'
Name of reference channel in txt files: '*Bead Drift Correction*'

TITRATIONdata

General description:
streptavidin-coated polystyrene particles (320 nm diameter) incubated with biotin-conjugated DNA docking strand at different concentrations; washed and imaged on glass slide in imaging buffer (5 Mm Tris-HCl, 10 mM $MgCl_2$, 1mM EDTA, 0.05% Tween-20, pH = 8) containing complementary DNA imager strand conjugated with Atto647N dye.
See table below for concentration values.

Acquisition parameters:
256x256 pixels region of camera, pixel size = 160nm
total internal reflection irradiation; T=25ºC
main excitation = 647 nm (20% of max power)
reference excitation = 561 nm (0.3% of max power), 1 frame every 100 of main-channel
camera exposure time = 100ms (10 Hz)
10000 or 15000 consecutive frames (see table below)
Max power 647 nm laser = 160 mW
Max power 561 nm laser = 80 mW

Analysis parameters in NIS-elements:
Camera baseline value = 100
Minimum width (nm) = 200
Maximum width (nm) = 700
Max. axial ratio = 1.3
Max. displacement (pix) = 1
minimum intensity threshold main channel = 700
maximum intensity threshold main channel = 20000
minimum intensity threshold reference channel = 500
maximum intensity threshold reference channel = 20000
time-trace length = 1-10 frames; first 700 frames excluded from analysis

Analysed data (TXT file name)	Raw data (TIFF file name)	Biotin docking concentration (nM)	Imager concentration (nM)	Main channel frames
2017-10-06_05nM_0004.txt	2017-10-06_05nM_0004.tif	0.5	5	10000
2017-10-25_5nM_0004.txt	2017-10-25_5nM_0004.tif	5	0.5	15000
2017-10-25_5nM_0005.txt	-	5	0.5	15000
2017-10-25_5nM_0006.txt	-	5	0.1	15000
2017-11-02_20nM_0001.txt	2017-11-02_20nM_0001.tif	20	0.1	15000
2017-11-02_20nM_0002.txt	-	20	0.1	15000
2017-11-02_20nM_0003.txt	-	20	0.1	15000
2017-10-13_50nM_0002.txt	-	50	0.1	10000
2017-10-13_50nM_0006.txt	-	50	0.1	15000
2017-10-25_50nM_0003.txt	2017-10-25_50nM_0003.tif	50	0.1	15000
2017-10-25_50nM_0007.txt	-	50	0.1	15000
2017-10-25_50nM_0008.txt	-	50	0.1	15000
2017-10-13_500nM_0003.txt	-	500	0.1	10000
2017-10-25_500nM_0002.txt	2017-10-25_500nM_0002.tif	500	0.1	15000
2017-10-13_6250nM_0004.txt	-	6250	0.1	10000
2017-10-25_6250nM_0001.txt	2017-10-25_6250nM_0001.tif	6250	0.1	15000

Name of main channel in txt files: '*647*'
Name of reference channel in txt files: '*Bead Drift Correction*'

Matlab Codes

<u>READCOORDS</u>: reads raw data from SMLM. Read raw data (TXT or CSV) obtained from SMLM analysis in NIS-elements (Nikon N-STORM) or ONI software, and extract coordinates of interest for further processing. It generates txt file of 3 XYT coordinates to use for further processing.

INPUTS:
FileName: name of the file with extension, e.g. 'MyFile.txt'
InputType: denotes the type of file, type 'N-STORM' for Nikon software TXT files, or 'ONI' for CSV files from ONI.

IMPORTANT: only two-channels can be read: main-channel (named 647) and fiducial markers channels (named Fid). File format should be checked. N-STORM files (TXT) are supposed to be 26-column, columns 4-5-13 are read as X-Y-T. ONI files (CSV) are supposed to be 11-columns, columns 3-4-2 are read as X-Y-T.

OUTPUTS:
X-Y-TCoords647: X, Y, T(frames) coordinates of localization in the main channel, named 647, expressed in nanometers
X-Y-TCoordsFid: X, Y, T(frames) coordinates of localization in the second channel (typically fiducial markers), expressed in nanometers
Two files in txt format containing X-Y-T coordinates of the two channels respectively

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<u>CLUSTER1</u>: perform clustering of (X,Y,T) coordinates with Mean-Shift algorithm using a SINGLE channel to identify clusters having particle-like shape with user defined selection parameters.

REQUIRED INPUTS:
FileName: name txt file with XYT coords, in nm and frame number
bandwidth: parameter for clustering, in nm
MinPts: minimum number of localizations in a cluster
MaxDiam: maximum diameter (longest axis), in nm

OPTIONAL INPUTS:
Elong: max ellipse elongation allowed, default=2.0
ScaleFactor: scale factor in ellipse fit, default=1.0
MinClustDist: minimum distance between clusters (in nm), default=300
AggrDist: minimum distance between clusters to be non-aggregates (in nm), default like MinClustDist
FracThreshold: fraction of cluster localizations within fitted sphere (default 0.9)

OUTPUTS:
Loc2particle: cell array with all info for each selected cluster
ClusSize: number of localizations in each selected cluster
diam: diameter of each selected cluster (in nm)

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<u>CLUSTER2</u>: perform mean-shift clustering of (X,Y,T) coordinates of REFERENCE channel in order to roughly identify centers of valid particles. Then, the localizations in MAIN channel within a defined distance from centers are stored.

REQUIRED INPUTS:
FileNameMain: txt file with XYT coords of main channel, in nm and frames
FileNameRef: txt file with XYT coords of ref channel, in nm and frames
bandwidth: parameter for clustering, in nm
MinPts: minimum number of localiz in a cluster
MaxDiam: maximum diameter (longest axis), in nm

Maxdistance: maximum distance between particle center and localization in main channel to be considered attached, in nm

OPTIONAL INPUTS:
Elong: max ellipse elongation allowed, default=10.0
ScaleFactor: scale factor in ellipse fit, default=1.0
MinClustDist: minimum distance between clusters (in nm), default=300
AggrDist: minimum distance between clusters to be non-aggregates (in nm), default like MinClustDist

OUTPUTS:
Loc2particleMain: cell array with XYT of MAIN channel for each selected cluster
C: XY coordinates of detected particles centers
ClustSize: for each selected cluster, number of counted localizations in the MAIN channel

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<u>QPAINT</u>: time-trace analysis for quantitative PAINT. The function takes the (X,Y,T) coordinates of localizations on each pre-selected NP or clusters, builds a binary time-trace and calculates the distribution of the dark times and their empirical cumulative distribution function. T is expressed as frame number.

INPUTS:
Loc2particle: a cell array containing the (X,Y,T) coordinates of localizations corresponding to each selected NP (T=frame number)
Nframes: total number of frames (including bead drift correction)
nbin: number of binnings for statistics
cut: number of initial frames to discard

OUTPUTS:
ParticleTimeTraces1: cell array containing the binary time-traces for each NP
dark_times: cell array containing all the individual dark time for each NP
dark_mean: mean values calculated from individual dark times for each NP
dark_median: median values calculated from individual dark times for each NP
CDF: cell array containing the empirical cumulative distribution function for each NP
dark_distribution: cell array containing the distribution of dark time intervals for each NP
cut_frames: number of intial frames not considered
emptyNP: numer of NP with no localizations in the range considered