3D-Image simulation of Platelet Receptor Distributions

The following MatLab scripts allow to create 2-color, 3D image stacks based on simulated platelet receptor distributions and the image resolution provided by confocal microscopy and sample expansion. Two scripts have to be executed sequentially:

- PlateletColocSimulation_P1_DummyPlatelet.m This script calculates a 3D point cloud of evenly spaced positions on the surface of a spheroid with defined lateral and axial radii.
- PlateletColocSimulation_P2_3DImageStack.m Based on the 3D point cloud created in 1., image parameters, expansion factor, defined receptor densities and clustering and colocalization parameters 2-color 3D image stacks are reconstructed.

Requirements:

- MatLab R2018b incl. Curve fitting toolbox
- interparc.m function: John D'Errico (2017). interparc (https://www.mathworks.com/matlabcentral/fileexchange/34874-interparc), MATLAB Central File Exchange. Retrieved November 27, 2017.
- points2srtfi3DNoise_Hh.m, Hannah S. Heil
- 1. Simulation of 3D point cloud of evenly spaced positions on the platelet surface
 - Step 1: Run PlateletColocSimulation_P1_DummyPlatelet.m
 - Step 2: Input via dialog:

a - lateral (x,y) platelet radius in nm

b - axial (z) platelet radius in nm

• Step 3: Output saved as .mat:

Dist - Distance between generated positions in nm.

AllPoints - .mat file with matrix AllPoints containing all generated equidistant receptor positions [x,y,z] on platelet surface in nm.



Figure 1: Defining the platelet geometry based on a spheroid with the lateral radius a and axial radius b. **a**) Scheme of the spheroid geometry, **b**) Input dialog for dummy platelet parameters.

2. Generation of platelet receptor distributions and 2-color 3D image stacks

- **Step 1:** Run PlateletColocSimulation_P2_3DImageStack.m
- Step 2: Define image parameters via dialog (Figure 2a & b):

Number of simulated stacks - Number of simulated stacks for each expansion factor

Distance between labels - Minimum distance between the two markers in the unexpanded case to account for the linking error and it's amplification during expansion (see also Figure 4 c)

PSF width (x,y) [nm] – lateral extension of the point spread function of the optical system in nm

PSF height (z) [nm] – axial extension of the point spread function of the optical system in nm

Voxelsize (x,y) [nm] – lateral voxelsize in the reconstructed 3D image in nm

Voxelsize (z) [nm] – axial voxelsize in the reconstructed 3D image in nm

Backgroundlevel [photons] – Number of photons contributing to the background level in photons

Std of Read Noise [photons] – Standard deviation of number of photons contributing to the read noise in photons

Noise calculation: Read noise (Gaussian distr. around 0) + Poisson distributed photon noise (sqrt(N)) + background level, no negative values are set to 0

承 P	_		×		
Number	of simula	ated sta	cks		
5					
Distance	betwee	n labels	[nm]		
0					
PSF wid	th (x,y) [r	nm]:			
250					
PSF heiç	ght (z) [n	m]:			
500					
Voxelsiz	e (x,y) [n	m]:			
40	40				
Vovelsiz	o (z) [com	J.			
180	e (z) (nn	ıj.			
100					
Intensity [photons]					
5000					
Expansio	onfactor:				
1,4,10					
	0	к	Cancel		
承 P	_		×		
Backgrou	undlevel	Inhotor	sl		
5		proof	-01		
Std of Re	ad Nois	e [phot	ons]		
-					

a)

b)

Figure 2: Image parameters

📣 Dummy Data							×
← → • ↑ <mark>.</mark> « A	nalysis > #Scripts > 201910_ExMSimu	ulation > PlateletColoc >	~	Ō		oloc" durchsuc	chen
Organisieren 👻 Neu	er Ordner						?
Dieser PC	Name	^			Änderungsdatum	Тур	
🗊 3D-Objekte	📙 Upload				15.10.2020 17:42	Dateiordne	r
📰 Bilder	PlateletColocSimulation_V1_Dur	mmyPlatelet_a-1500nm_b-500nm_Dist-1.6723n	m_Workspace.r	nat	25.10.2019 16:38	MATLAB Da	ata
E. Desktop							
🛫 users (\\132.187.							
_ ¥	·						
Date	iname: PlateletColocSimulation_V1_Du	mmyPlatelet_a-1500nm_b-500nm_Dist-1.6723n	m_Workspace.	mat 🕓	MAT-files (*.ma	t)	\sim
					Öffnen	Abbrech	eni

Figure 3: Importing 3D point cloud on platelet surface from matlab workspace data created with PlateletColocSimulation_P1_DummyPlatelet.m.

- Step 3: Load 3D point cloud coordinates from Dummy Platelet data created with script 1 (Figure 3)
- **Step 4:** Select receptor distribution patterns (Figure 4a) and colocalization test case (Figure 4b):



Figure 4: Dialog selection of a) receptor distribution pattern and b) colocalization mode.

- **A.** Single Receptors:
 - Randomly distributed single receptor positions for marker A and B (Figure 5 a)
 - In Colocalization Test Case 1 "Maximum Coloc" both marker occupy the same positions only separated by the "Distance between labels" d as defined in Step 1 (Figure 5 a, zoom-ins).
 - In Colocalization Test Case 2 "Minimum Coloc" each position can only be occupied by either marker.
 - The total number of receptors per platelet for marker A and B and the marker retention ratio in % is defined in a dialog (Figure 5 c)
- **B.** Receptor Clusters:
 - The marker positions are distributed in clusters (Figure 5 b), with the Cluster density D_c, the Cluster Area A_c and the Number of Receptors per Clusters N_R.
 - The cluster parameters and the marker retention ratios are defined in a dialog (Figure 5 d).
 - The colocalization test are already described in A.
- **C.** Partial Colocalization:
 - Randomly distributed single receptor positions for marker A and B (Figure 5 a)
 - The total number of receptors per platelet for marker A and B, the marker retention ratio in % and the ratio of colocalization R_{coloc} in % is defined in a dialog (Figure 5 e)
 - While the Ratio of R_{coloc} of the marker positions are occupied by both markers only separated by the "Distance between labels" d as defined in Step 1 (Figure 5 a, zoom-ins), the remaining position can only be occupied by either marker.

a)	b)				
Mi	in. Max. loc. Coloc.				
c)	d)	e)			
	\land Plat — 🗆 🗙				
	Mean Cluster Area Receptor A [nm^2]:				
	Mann Cluster Area Reporter B [ami/2]:	承 P — 🗆 🗙			
💽 P — 🗆 🗙	300	Number of Receptor A:			
Number of Receptor A:	Localzations per Cluster Receptor A:	120000			
120000	7	Number of Receptor B:			
Number of Receptor B:	Localzations per Cluster Receptor B:	120000			
120000	7	Retention Ratio A [%]:			
Retention Ratio A [%]:	Cluster Density Receptor A [um^-1]:	100			
100	70	Retention Ratio B [%]:			
Retention Ratio B [%]:	Cluster Density Receptor B [um^-1]:	100			
100	70	Ratio of Colocalization [%]			
OK Cancel	Retention Ratio A [%]:	30			
OK	60	OK Casad			
	Petertion Patio B [%]	UK Cancel			
	60				
	OK Cancel				

Figure 5: Receptor distribution patterns and parameters for the three distribution modes. a) Random distribution of single receptor positions and marker positions in the Colocalization Test Case 1 "Maximum Coloc" and Test Case 2 "Minimum Coloc". b) Position distribution for receptor clusters with a defined cluster density D_c, cluster area A_c and number of receptors per cluster N_R. c-e) Parameter input dialogs for the different receptor distribution patterns: c) "Single Receptors", d) "Receptor Clusters" and e) "Partial Colocalization".

• Step 5: After randomly excluding marker positions based on the defined retention ratio, a 3D image stack is reconstructed for each colour channel based on the function points2srtfi3DNoise_Hh.m and saved as .tiff stack.