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:

1. 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.
2. 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

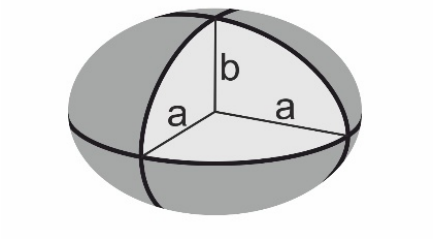
- **Step 1:** Run PlateletColocSimulation_P1_DummyPlatelet.m
- **Step 2:** Input via dialog:

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.

a)



b)

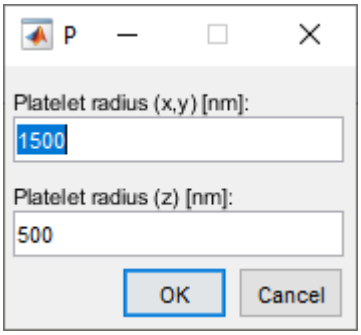


Figure 1: Defining the platelet geometry based on a spheroid with the lateral radius a and axial radius b. **a)** Scheme of the spheroid geometry, **b)** Input dialog for dummy platelet parameters.

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

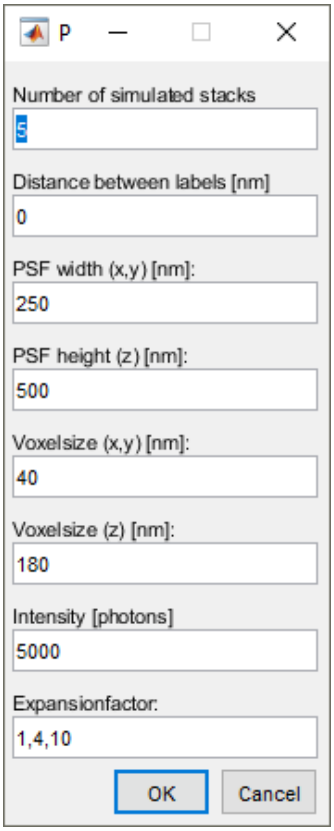
  Voxelsize (z) [nm] – axial voxelsize in the reconstructed 3D image in nm

  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

a)



b)

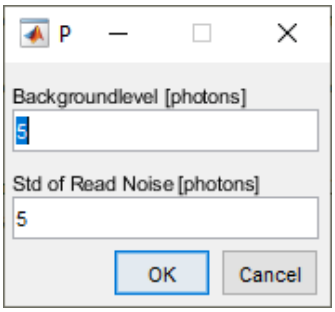


Figure 2: Image parameters

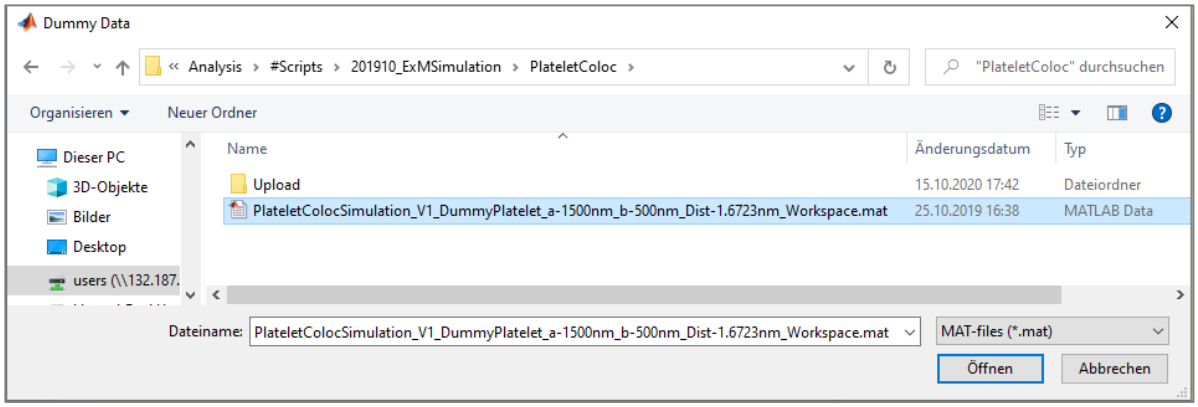


Figure 3: Importing 3D point cloud on platelet surface from matlab workspace data created with PlateletColocSimulation_P1_DummyPlatelet.m.

- **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):

a) **b)**

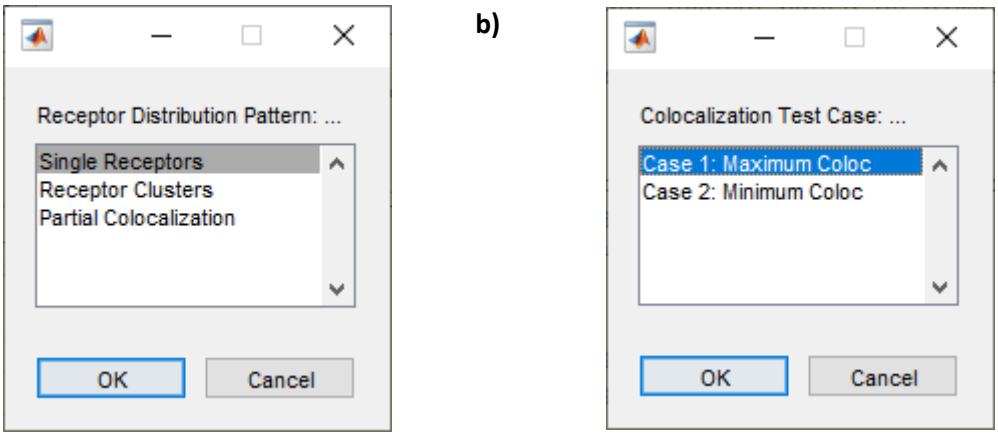


Figure 4: Dialog selection of **a)** receptor distribution pattern and **b)** colocalization mode.

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.

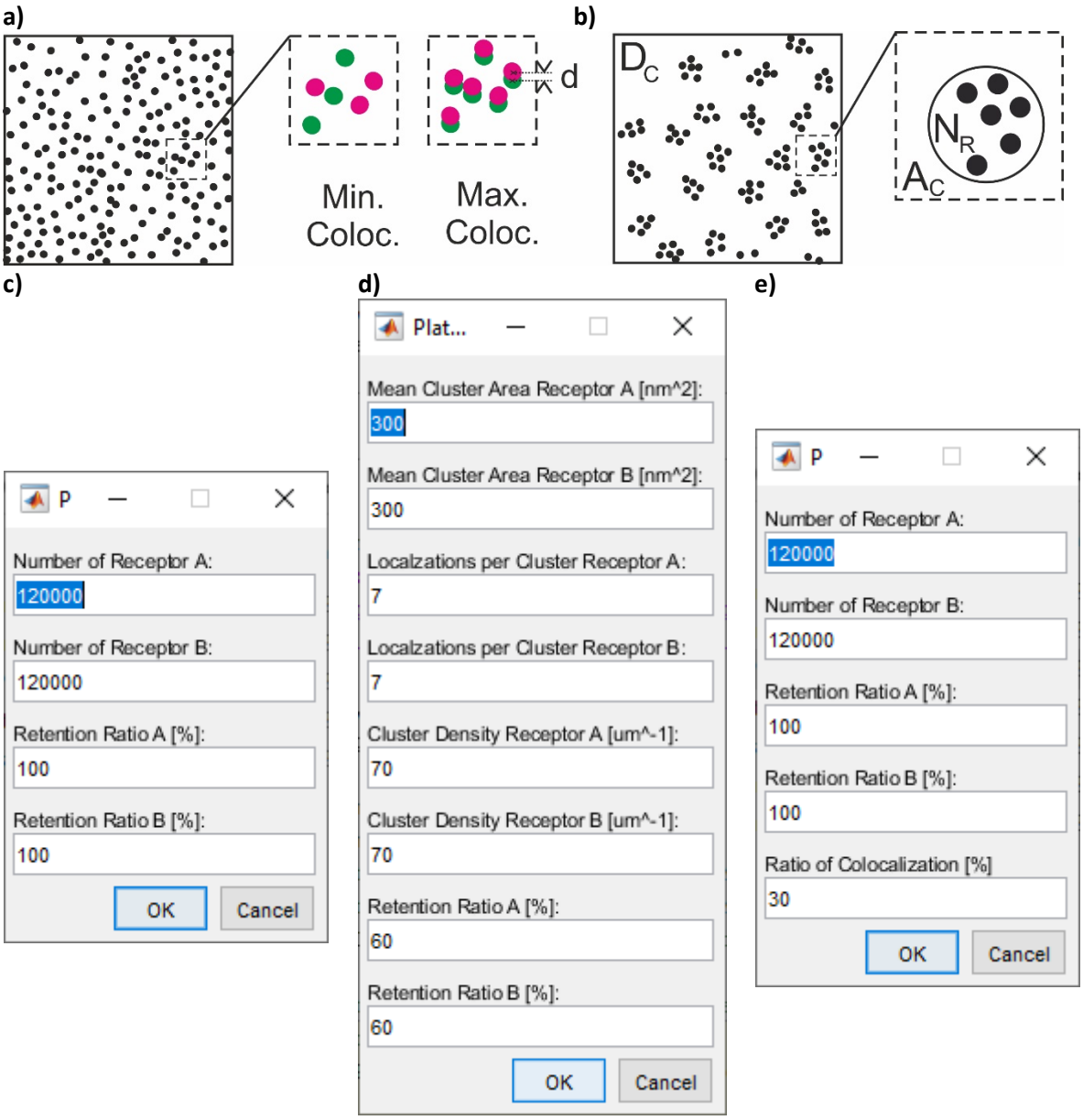


Figure 5: Receptor distribution patterns and parameters for the three distribution modes. a) Random distribution of single receptor positions and marker positions in the Colocalization Test Case 1 "Maximum Coloc" and Test Case 2 "Minimum Coloc". **b)** Position distribution for receptor clusters with 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.