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Application of Artificial Intelligence Strategies to the Analysis of Neurotransmitter Receptor Dynamics in Living Cells

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STORM (stochastical optical reconstruction microscopy), a form of single-molecule nanoscopy, calls for a variety of statistical and mathematical operations to reconstruct the original objects from their noisy wide-field point spread functions [1]. We are interested in understanding the dynamics of the nicotinic acetylcholine receptor (nAChR) protein, a cell-surface neurotransmitter receptor. Analyzing the translational motion of nAChR molecules by single-particle tracking in living cells is a complex task. In order to understand how nAChR molecules associate/dissociate into/from nanometer-sized clusters over time, and to characterize their trajectories according to different mathematical models, we are developing analytical procedures based on artificial intelligence. Due to their speed of calculation and accuracy, deep learning models are clearly an improvement on classical models in biological image analysis and biomedical science.

The analysis of translational motion at the ensemble level is generally done by applying mean-square displacement (MSD) methods, which rely on the average spatial distances between a given trajectory measured between increasingly long time-lags. Instead, we chose to apply a novel strategy to characterize particle diffusion based on Deep Learning (DL) methods [2]. Prior to applying this procedure to the actual experimental data, we trained the system using simulated data representing several physical models of translational motion often followed by diffusing macromolecules. Granik's analysis [2] discriminates between the so-called fractional Brownian motion (fBm), the canonical thermally-driven Brownian motion, and the continuous-time random walk (CTRW) type of motion. The strategy is summarized in the flowchart below:

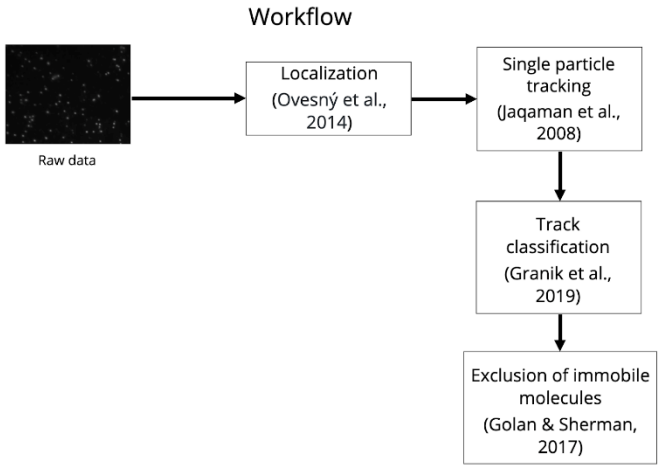

Figure 1. Workflow. In order to classify the motional regimes followed by nAChR molecules it is first necessary to establish the x,y coordinates ("localization") of the molecules, using the ImageJ plugin ThunderSTORM. Next we determined the single-molecule trajectories, using Jaqaman et al.´s software in MATLAB. We next fed the experimental data to the previously trained DL network with the simulated data.

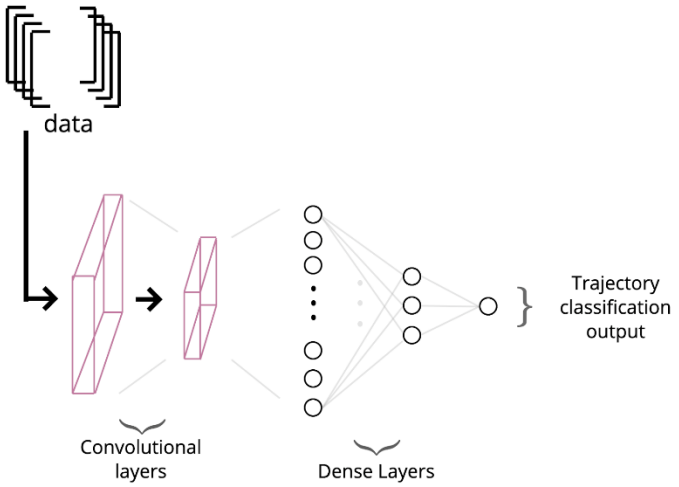

Figure 2. Trajectory classification by Deep Learning. Convolutional layers and dense layers are the main components of the network. Input data consists of (x,y) localization coordinates and a track identifier. The first component of the network is a convolutional concatenation of 4 layers, followed by a subsequent set of 3 dense layers. The last dense layer in turn consists of 3 nodes, each corresponding to a different diffusion model (fBm, Brownian or CTRW). The classification output is determined by the highest rated node in the last dense layer.

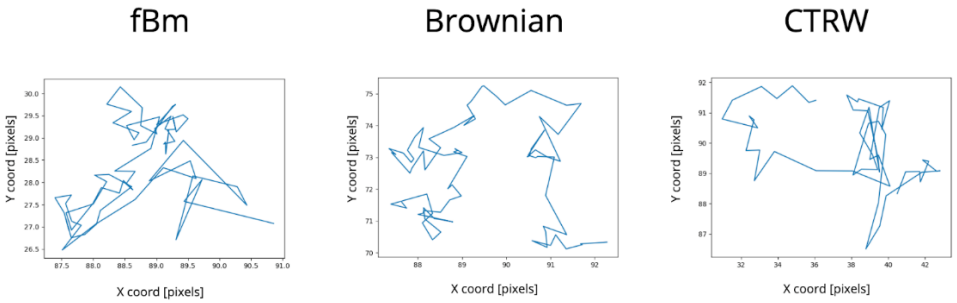

Figure 3. The plots correspond to the outputs of the Granik et al. [2] analysis as applied to the actual experimental data of nAChR trajectories. Fractional Brownian motion (fBm), also known as fractal Brownian motion, is a Gaussian process which differs from pure Brownian motion in that the increments of the process are normally distributed but not independent. Brownian motion represents the thermally-driven motional regime followed by a large variety of membrane-associated proteins. The continuous-time random walk (CTRW) type of motion is also a stochastic walk process, where the moving particle waits for a random time between steps.

In conclusion, application of artificial intelligence approaches enabled us to classify the nAChR trajectories according to three physical models of diffusion. The motional behavior of cell-surface nAChRs is characterized by its heterogeneous nature. The proportion of trajectories undergoing a certain motional regime changed upon modifying the cholesterol concentration in the membrane, as in previous studies from our laboratory [3], [4].

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