Seeing is believing – a reverse simulation approach assessing the ability of 3D localization microscopy to resolve defined nanoscopic structures

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KEY WORDS: superresolution microscopy, 3D localization microscopy, electron tomography, simulation, image resolution

ABSTRACT:
3D localization microscopy techniques have been increasingly employed to circumvent the diffraction limit and to uncover molecular organizations and novel structures on the nanoscopic level. Several methods have been developed to determine localization precision and image resolution without a priori knowledge of the underlying structure [1-3]. However, these measures do not directly address the question if localization data can faithfully reconstruct distinct 3D geometries of structures of interest intuitively deemed accessible to localization microscopy. For example, can short filamentous structures irregularly arranged in space be adequately resolved? To address this question, we developed a reverse simulation tool that predicts the 3D localization pattern based on models representing the ground truth. The latter was obtained from electron tomographic data or generated from assumed distributions of molecular structures. The simulation considers variables such as the distance between label and object of interest, the distribution of binding angles of antibodies, the number of binding sites per surface area, labeling efficiency and depth distribution in a certain volume, localization precision and average blinking frequency per fluorophore. By varying these parameters systematically the best-possible localization images can be simulated for ground truth models. This approach allows microscopists to predict if a given nanostructural feature can be resolved using 3D localization microscopy. We first tested this reverse simulation approach using electron tomographic reconstructions of several organelles. In all cases examined, the reversely simulated images closely resembled the published images taken with 3D localization microscopy, suggesting that reverse simulation produces reliable predictions. We then tackled the notoriously difficult question of actin filament organization in presynaptic terminals. This was done by manually segmenting structures representing filamentous actin from electron tomograms of the calyx of Held, a mammalian excitatory model synapse. 3D localization images were then calculated using realistic parameters obtained from the literature. The simulated images were in remarkable agreement with images obtained using 3D dSTORM imaging of phalloidin-Alexa 647-labeled calyx terminals. Evidently, the precise 3D localization of short filamentous actin cannot be resolved with current 3D localization microscopy techniques. Even when running the simulation with an optimal parameter set including a label distance of 1 nm, the nanarchitecture of actin could not be resolved.

In conclusion, we developed a simple tool to determine the applicability of 3D localization microscopy to a biological question based on an assumed or experimentally determined ground truth.