

Probing identified synapses at the nanoscale: developing software tools for visualization and analysis of correlated confocal- and STORM super-resolution microscopy

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Introduction

Understanding the molecular mechanisms underlying physiological and pathophysiological processes of the nervous system requires adequate methods to determine the nanoscale targeting of proteins within neurons. The power of high-resolution light- and electron microscopic techniques is often limited by the fact that brain circuits are comprised of several highly compartmentalized cell types. STochastic Optical Reconstruction Microscopy (STORM) is a flexible and efficient way to obtain precise molecule localization data. Combining STORM with confocal microscopy to visualize identified cells opens the ability to analyze super-resolution data in a cell type- and compartment-specific manner. However, while advanced tools exist for the handling of either confocal or STORM image data, to date, the correlated visualization and analysis of both is not available. Moreover, the localization-based data from STORM allows for a wide variety of analysis approaches that are impossible to perform on conventional, voxel intensity-based microscopy data, and, thus, are not included in available software packages.

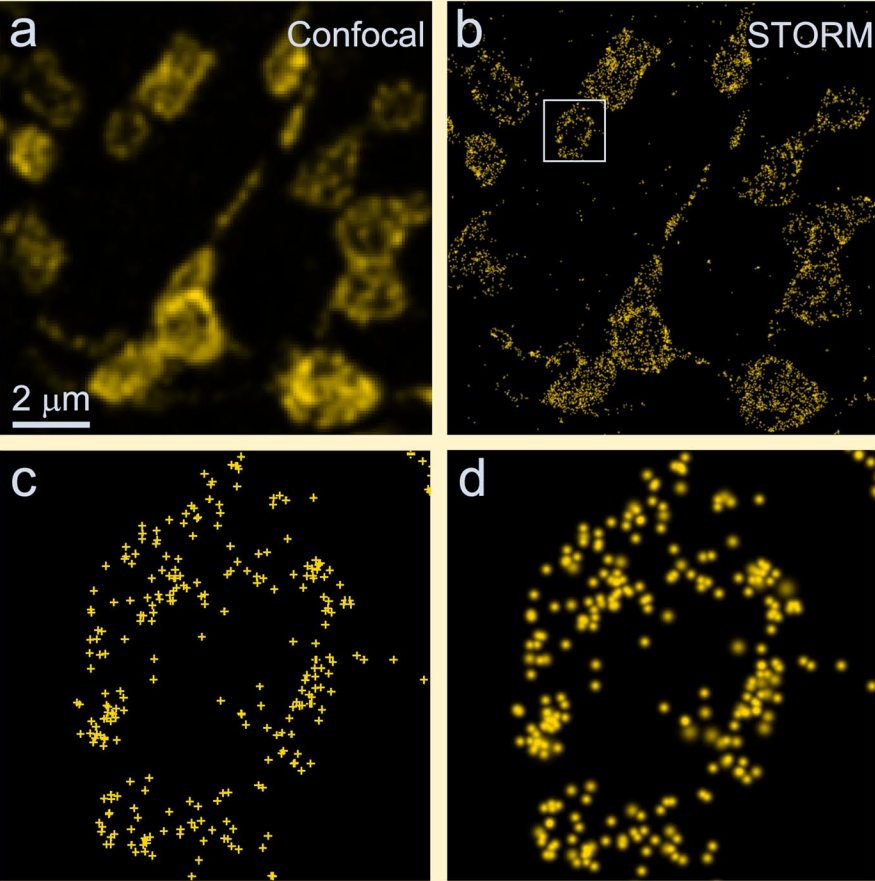
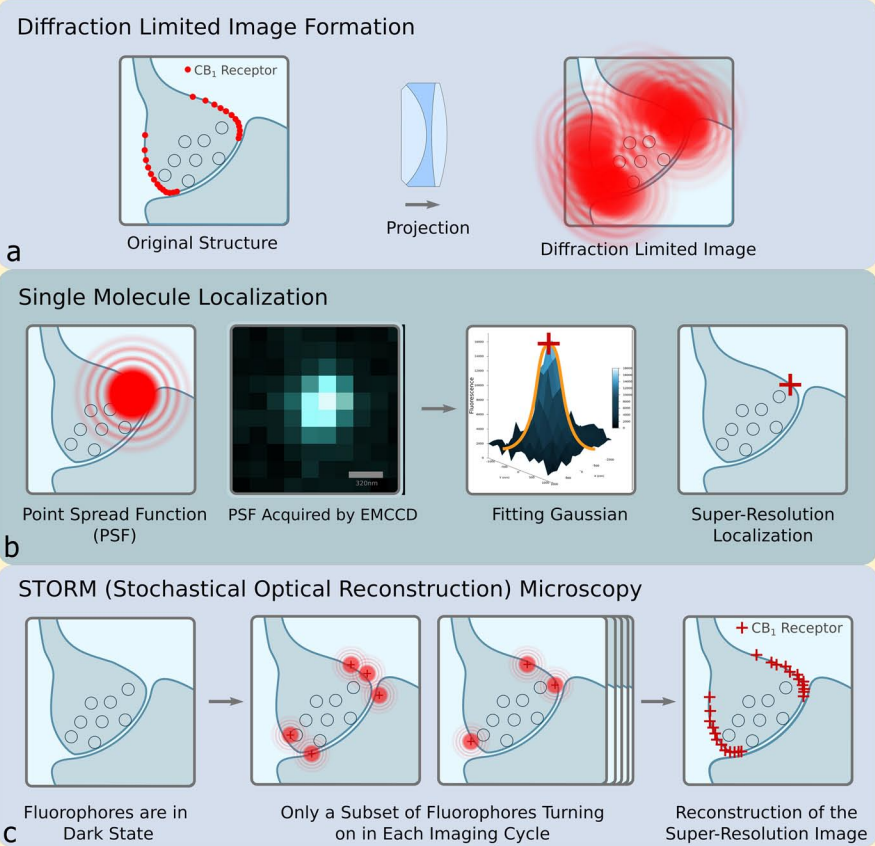


Figure 2. Visualization of confocal and STORM data with the NIS-Elements software

a, Deconvolved confocal image of immunostaining against CB₁ receptors (G protein-coupled receptors, localized in the plasma membrane) in the CA1 region of the hippocampus.
b, The same field of view imaged by STORM microscopy.
c, Localization points representing CB₁ receptors on a single axon terminal. Since the data obtained with STORM is not a conventional pixel intensity-based image, but the list of coordinates of localization points, LPs are represented as crosses.
d, Based on the peak height of the LPs in the detection frame, each can be visualized with Gaussian spots with different full width at half maximum (FWHM) values, representing localization precision. Higher Gaussian intensity corresponds to better localization precision. This visualization method provides an image which is easier to evaluate visually.

Aims

However NIS-Elements is an advanced tool for handling STORM and confocal images it has some limitations that hinders the convenient analysis of STORM data. It lacks the ability of parallel visualization of confocal images and STORM localization points thus correlated analysis of those is not possible. An other issue is that custom subpopulations of STORM LPs cannot be isolated for further tasks.

Therefore, we aimed to implement the above features in a custom-made program designed for the needs of molecular neuroscience. We developed an easy-to-handle graphical user interface in MATLAB, which we employed to determine the nanoscale localization of molecular components of the endocannabinoid system within axon terminals of identified neurons.

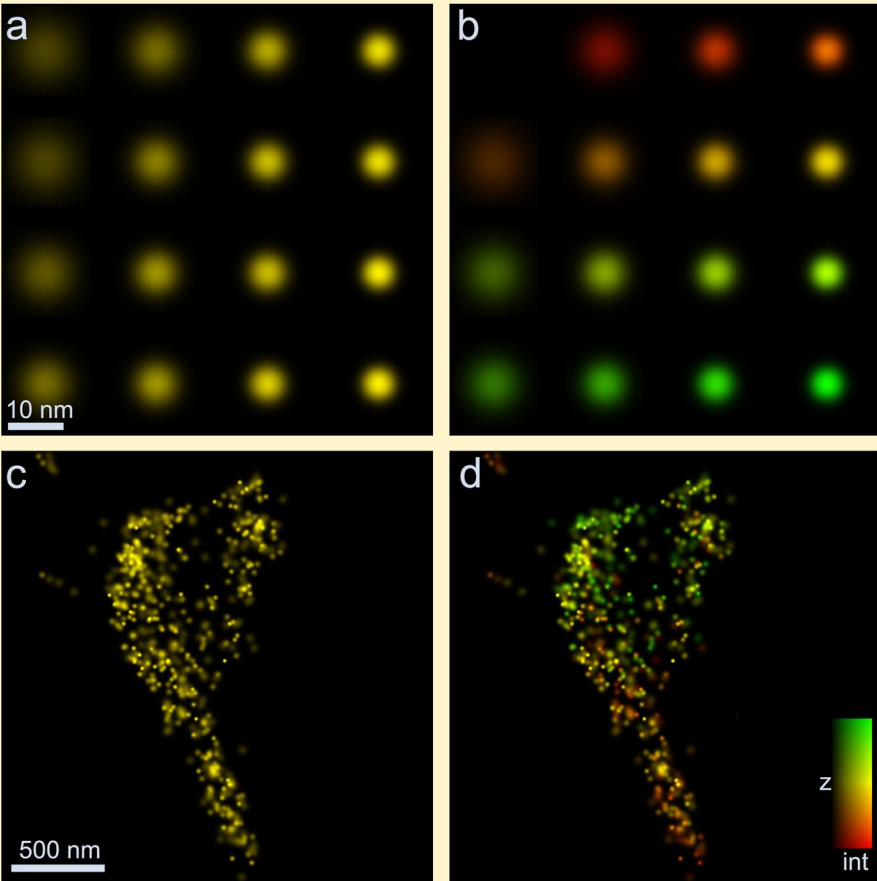


Figure 3. STORM data representation with our custom-made software

a, Visualization of LPs with different localization precision. FWHM value of the Gaussian spots corresponds to the localization precision of each point.
b, Visualization of LPs with different z coordinates (-400 to +400 nm from the focal plane) and localization precision using z color coding.
c, Representation of a STORM image of anti-CB₁ receptor immunostaining with channel coding.
d, Representation of the same axon terminal with z color coding.

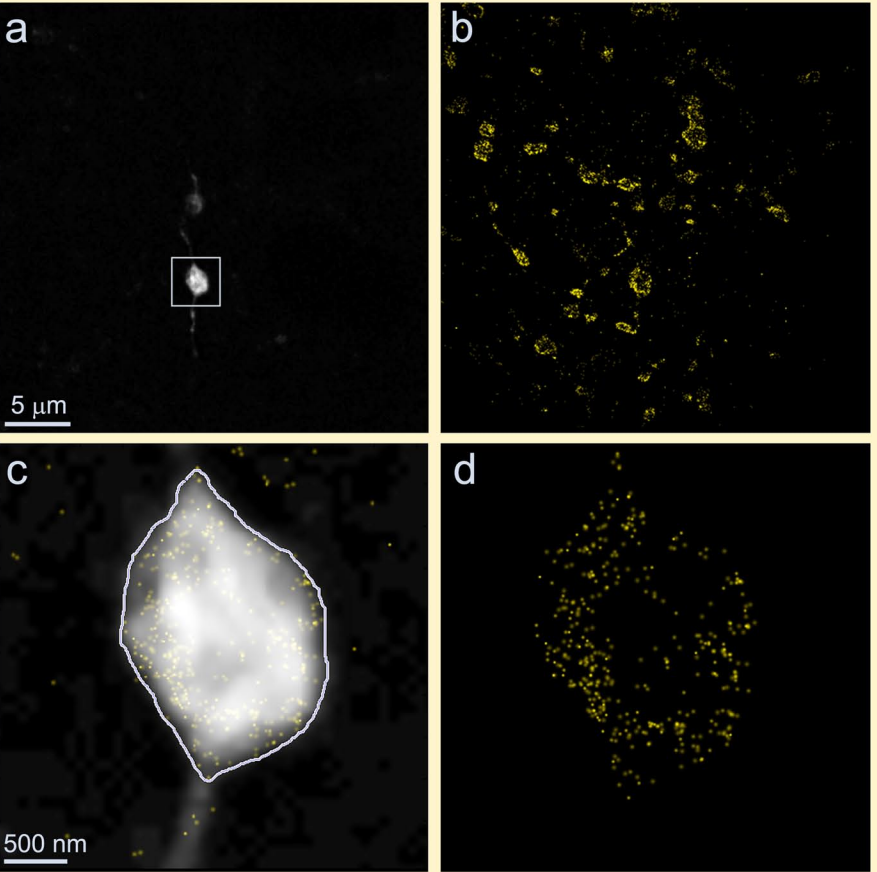


Figure 4. Cell- and subcellular domain-specific analysis of correlated STORM and confocal data

a, Deconvolved confocal image of an axon of an identified, biocytin-filled hippocampal CA1 interneuron.
b, STORM image of anti-CB₁ immunostaining in the same field of view.
c, Correlated CB₁, STORM data and confocal image of a biocytin-filled axon terminal. Localization points belonging to an individual axon terminal are selected based on the correlated confocal image of the bouton. The coordinates of LPs can be shown and saved for the purpose of subsequent analysis.
d, CB₁, STORM LPs defined by the freehand selection.

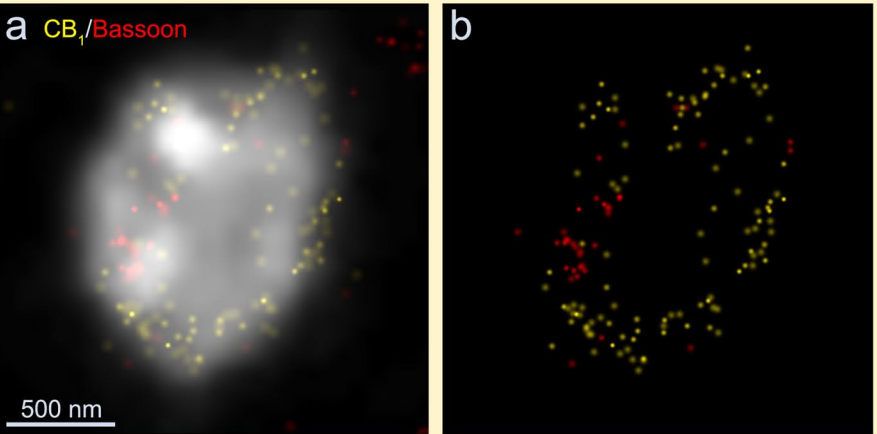


Figure 5. Handling of multi-channel STORM data

a, Correlated CB₁ (yellow) and bassoon (red, marker of synaptic active zones) immunostaining STORM data and deconvolved confocal image of a biocytin-filled axon terminal.
b, STORM LPs defined by a freehand selection, color coded by channel.

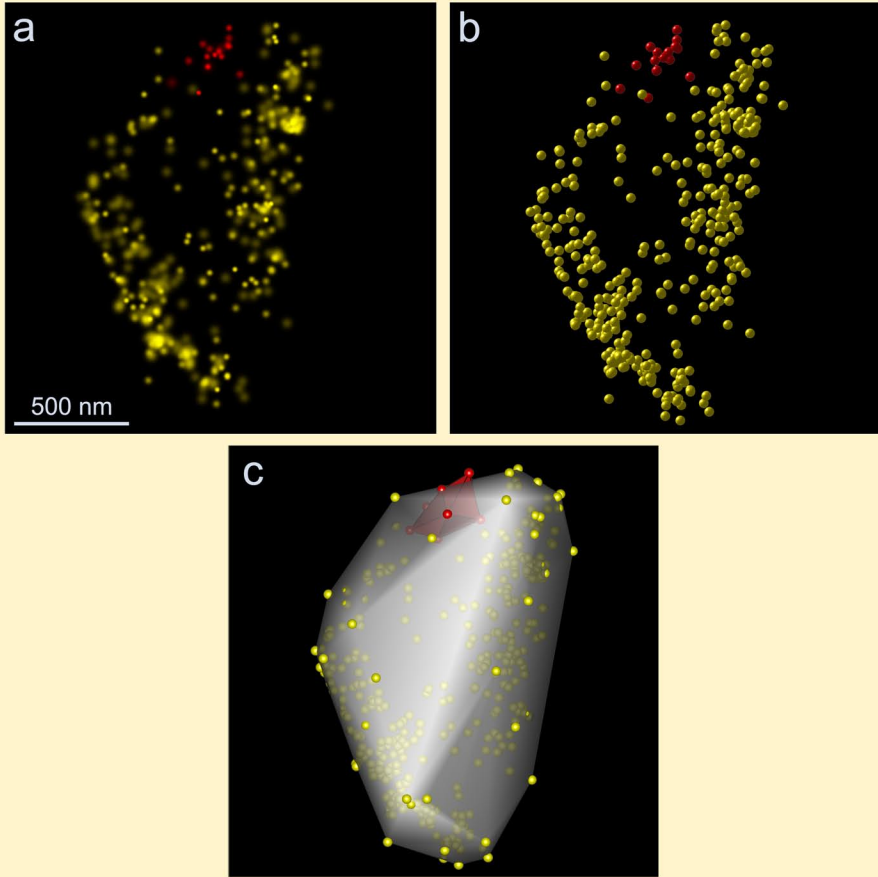


Figure 6. 3D visualization of STORM data

a, Double STORM image of Bassoon (red) and CB₁ (yellow) localization points on an axon terminal. The accumulation of bassoon indicates the presence of a synaptic junction.
b, The CB₁ and Bassoon localization points belonging to the axon terminal and the synapse, respectively, were selected, and processed for 3D visualization. LPs are represented as uniform spheres of arbitrary size.
c, The same localization points of bassoon and CB₁ groups were connected to form convex hulls (Bassoon - red, CB₁ - gray).

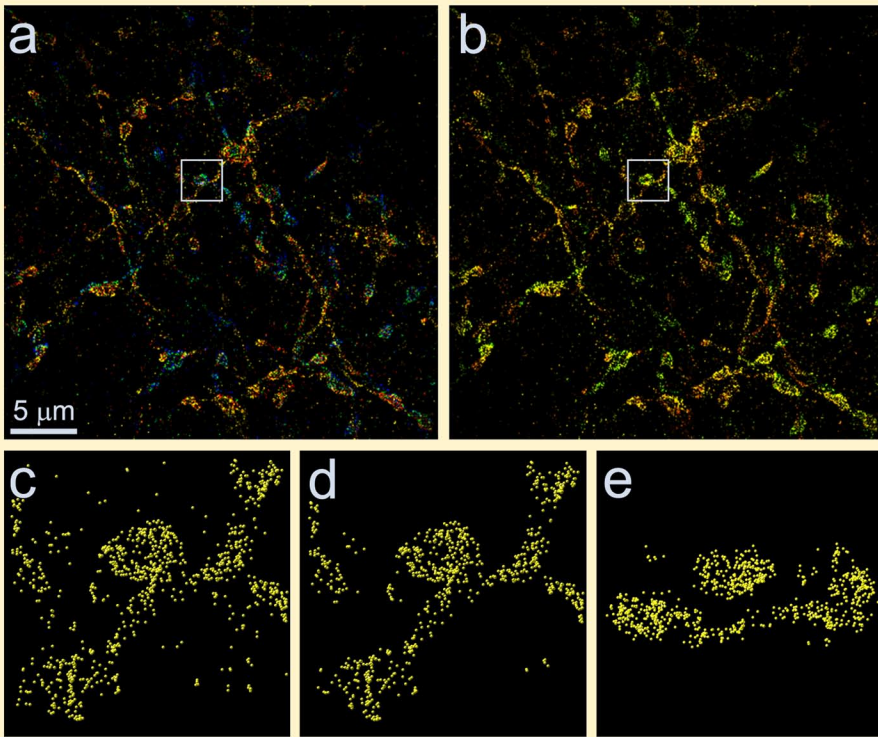


Figure 7. Visualization and processing of STORM z-stacks

a, A z-stack of STORM images of anti-CB₁ immunostaining acquired by imaging four, partially overlapping images along the z dimension. The focal planes of the consecutive images are 300nm apart.
b, STORM image of the same field on view visualized by z color coding.
c, Axon terminals and an axon in x-y view in 3D representation surrounded by small clusters of CB₁ LPs.
d, With the application of a local density-based filtering algorithm the small clusters were eliminated and only the axon terminals and the axon are visualized.
e, The axon terminals and the axon from a different angle in 3D representation. With the help of STORM stack processing and 3D visualization it becomes clearly visible that the axon is situated below the axon terminal in the center of the image.

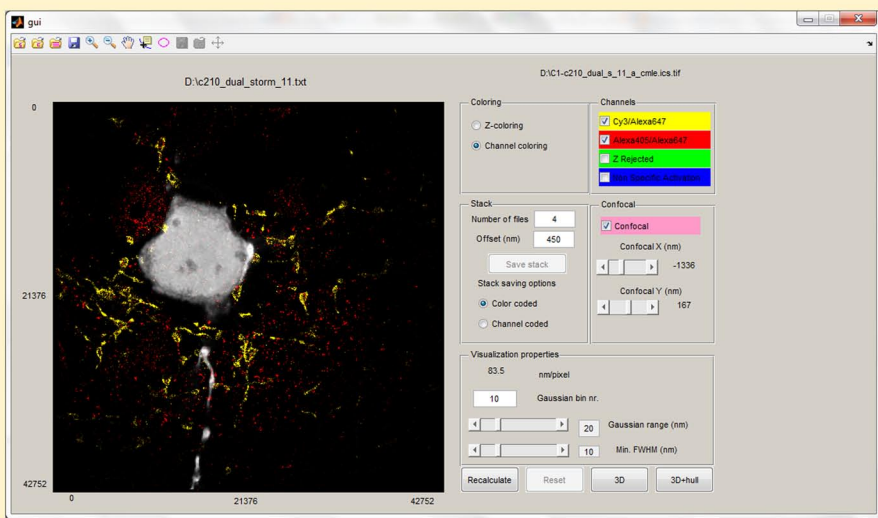


Figure 8. Graphical user interface of the custom-made visualization program

The program can open STORM text files and confocal images, and both the visualized images and STORM coordinates can be saved in multiple formats. Custom zoom and pan functions are implemented and custom selection of STORM data can be achieved. The image can be colored based on the STORM channels or the z coordinates. The width, intensity and number of the Gaussian spots is variable and the confocal image can be adjusted to the STORM image. The program can handle STORM stacks and the z-offset is variable.

Future Plans

- multipane view of 3D data
- 3D selection tool
- 3D confocal data handling
- implementation of batch STORM file analysis
- implementation of analysis tools
- advanced z-stitching
- optimization of image processing time

Acknowledgements:

