

Authors: *V. MICZÁN^{1,2}, L. BARNA¹, B. DUDOK^{1,3}, A. HORVÁTH², J. R. GLAVINICS^{1,2}, Z. I. LÁSZLÓ^{1,3}, I. KATONA¹;

¹Momentum Lab. of Mol. Neurobio., Inst. of Exptl. Medicine, HAS, Budapest, Hungary; ²Fac. of Information Technol. and Bionics, Pázmány Péter Catholic Univ., Budapest, Hungary; ³Sch. of Ph.D. Studies, Semmelweis Univ., Budapest, Hungary

Abstract: Super-resolution light microscopy techniques can visualize the nanoscale molecular composition of brain circuits with similar localization precision of target proteins such as immunogold electron microscopy. Moreover, the possibility to simultaneously visualize multiple proteins, together with the faster tissue processing protocols and the higher immunolabeling density represents potential major advantages over electron microscopy in several neuroscience applications. Indeed, we have recently shown that combining Stochastic Optical Reconstruction Microscopy (STORM) with confocal microscopy allows rapid and efficient nanoscale molecular imaging within morphologically and neurochemically defined cellular and subcellular context in intact brain circuits (Dudok et al., 2015, Nature Neuroscience, 18:75-86). To further facilitate the correlated visualization and data analysis of super-resolution and confocal images, we have developed the open-source software VividSTORM, and recently implemented multiple new features not yet available in any other microscopy software packages. VividSTORM offers the simultaneous, correlated visualization and analysis of both pixel-intensity-based (such as widefield, TIRF, confocal, STED or SIM) and coordinate-based (such as STORM or PALM) microscopy data. By using the graphical user interface of VividSTORM, the key steps of the correlated analysis of the two different imaging modalities are: i) to overlay the pixelated image and the single-molecule localization coordinates; ii) to outline the labelled target cell or subcellular structure on the conventional microscopy image as region-of-interest (ROI); iii) to filter those specific single molecule localization points which belong to the identified target profile; iv) to perform coordinate-based analysis of the super-resolution imaging data. We implemented several novel tools in VividSTORM in order to facilitate this process including: manual and automatic, fiducial-marker-based or image-based alignment methods; an unbiased ROI selection feature by automated 2D or 3D segmentation of the pixelated image using a modified Morphological Active Contours Without Edges (MACWE) algorithm; and the analysis of molecular clustering by using multi-scale Bayesian or DBSCAN methods. The batch analysis of pre-selected ROIs is also supported to further facilitate high-throughput correlated confocal and super-resolution data analysis and functional interpretation of quantitative molecular observations within identified cellular and subcellular structures.

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