"Project Pattern" - an open access online tool for spatial analysis of immunolabeling in electron microscopy.

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In biological research, the term "colocalization" is usually used in the context of fluorescence microscopy. However, the character of analysed data such as signal leakage from different channels, signal originating from other focal planes and low resolution as well as subjective thresholding may lead to erroneous conclusions. On the other hand, immunolabeling in electron microscopy can be used to detect structures and molecules with much higher precision than even novel super-resolution light microscopy techniques while sharing common principles with fluorescence immunolabeling. So far, the evaluation of such images was often subject to similar woes, lacking unbiased quantitative approach despite higher resolution provided by EM.

We are developing an easy-to-use online tool for semi-automatic multi-stage analysis of immunolabeling on EM images spanning particle detection and classification, mathematical and statistical evaluation and visualization of results that builds on previous work of A. A. Philimonenko et al. [1] and C. Schofer et al. [2]. The tool detects and classifies particles automatically after marking few individual particles of each class; results of identification can then be manually reviewed and edited. Spatial relations can be analysed in 2-dimensional and 3-dimensional microscopic data and along linear structures such as membranes and filaments. The tool uses pair correlation and pair cross-correlation functions for clustering and colocalization (Fig. 1) evaluation with results presented both numerically and graphically. Labelled structures are visualised via mapping and their spatial relations to other structures or particles are further evaluated as shown by V. V. Philimonenko et al. [3]. Statistical significance of detected patterns will be calculated and presented to the user without the need for deep insight into statistical analysis. All results and respective setting can be exported and particle coordinates can be kept on the server for prolonged periods of time to be reused with new settings or compared to new datasets. Projects can be shared with colleagues and reused with new data. For routine analysis, useful results should be obtainable in just few mouse clicks.

This online tool will be provided by the Institute Molecular Genetics of Czech Academy of Science to the broad scientific community in open access mode within the Czech-BioImaging research infrastructure. The tool will emphasize convenience and understandability of the interface and will provide detailed explanations of all results, steps, values and options. The platform will be modular and can be expanded with more capabilities in the future.

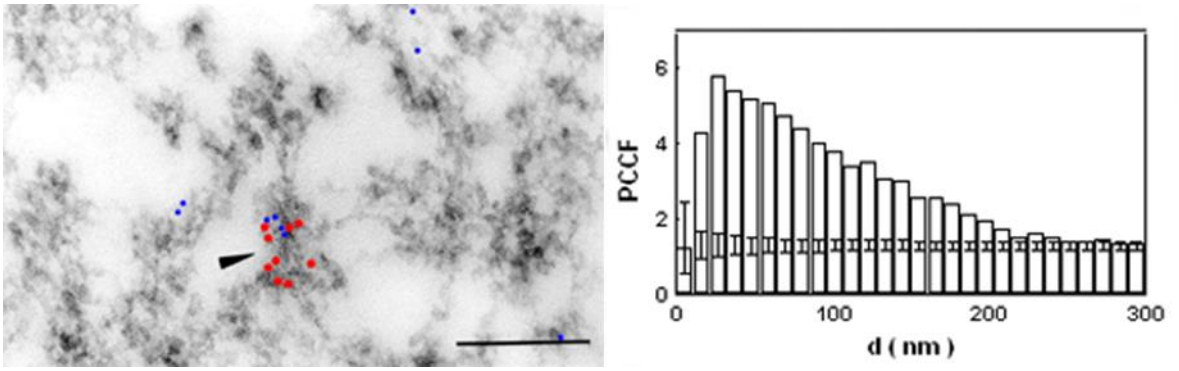

Fig.1: An Example of colocalization (arrowhead) of two antigens (marked with 10nm and 5nm gold nanoparticles - red and blue dots respectively). Values of PCCF well above 1 in the interval of 20-175 nm colocalization within this range as shown in histogram.

[1] Philimonenko, A. A., JanáÄek J. and Hozák P. "Statistical evaluation of colocalization patterns in immunogold labeling experiments." Journal of structural biology 132.3 (2000): 201-210.

[2] Schöfer, C., JanáÄek, J., Weipoltshammer, K., Pourani, J., & Hozák, P. (2004). Mapping of cellular compartments based on ultrastructural immunogold labeling. Journal of structural biology, 147(2), 128-135.

[3] Philimonenko, V. V., Philimonenko, A. A., Šloufová, I., Hrubý, M., Novotný, F., Halbhuber, Z., ... & Hozák, P. (2014). Simultaneous detection of multiple targets for ultrastructural immunocytochemistry. Histochemistry and cell biology, 141(3), 229-239.

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