NANOJ-SQUIRREL: QUANTITATIVE MAPPING AND MINIMISATION OF SUPER-RESOLUTION OPTICAL IMAGING ARTEFACTS

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Most super-resolution microscopy techniques depend on steps that can contribute to the formation of image artefacts, leading to misinterpretation of biological information. We present NanoJ-SQUIRREL, an open source ImageJ-based analytical approach that provides quantitative assessment of super-resolution image quality, capable of guiding researchers in optimising imaging parameters. By comparing diffraction-limited images and super-resolution equivalents of the same acquisition volume, this approach generates a quality score and quantitative map of super-resolution defects. To illustrate its broad applicability to super-resolution approaches we apply our method to Localization Microscopy, STED and SIM images of a variety of in-cell structures including microtubules, poxviruses, neuronal actin rings and clathrin coated pits. We particularly focus on single-molecule localisation microscopy, where super-resolution reconstructions often feature imperfections not present in the original data. By showing the quantitative evolution of data quality over these varied sample preparation, acquisition and super-resolution methods we display the potential of NanoJ-SQUIRREL to guide optimization of super-resolution imaging parameters.