

QUANTITATIVE SINGLE MOLECULE LOCALIZATION MICROSCOPY VISUALIZATION

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1. BACKGROUND

A number of approaches have been brought forward for the quantitative visualization of single molecule localization microscopy data. Extraction or estimation of the local emitter density facilitates comparison with other linear, intensity based microscopy approaches. Other methods aim at including additional quantities into the rendering process such as e.g. the localization precision or the local resolution. All methods are known to be affected from local fluctuations of the blinking behavior of the fluorophores, but presently quantitative evaluation of how the visualization strategies are affected by repetitive detection of the same molecule is – to a large degree – still unexplored.

2. RESULTS

We systematically studied various visualization strategies in respect to their influence on the choice of fluorophore/embedding media. While potentially fluorophores which exhibit a large recall, i.e. a high number of re-appearances, provide a better rendering of non-continuously labeled structures, it is possible to obtain quantitative intensity information from measurements employing fluorophores with a low recall. In line with previously reported results, our findings confirm, that fluorophores with a high recall rates allow for a reduction of the labeling density. We present results on the effect of recall on resolution obtained in the various visualization approaches. The comparison encompasses standard visualization methods such as point blurring, kernel density estimation, triangulation, and histogram binning as well as the recently employed technique of jittered grid binning [1]. Compared to approaches in which the individual positions are jittered around the extracted signal position (which is slightly offset from the true emitter position), the latter technique has the advantage that it does not add an additional error to the list of positions. Our results add to the ongoing debate on the structural resolution of SMLM visualizations.

3. REFERENCES

[1] A. Szczurek et al. *Nucleic Acids Research* doi:10.1093/nar/gkw1301 (2017).