The Quest for Perfection in Single Molecule Localization Microscopy

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Single Molecule Localization Microscopy (SMLM) has been increasingly popular since the technology was demonstrated on live and fixed cells in 2006 by three groups simultaneously [1,2,3]. SMLM has since then gained a third dimension, through either PSF engineering, interference or multi-focus acquisition. Labeling techniques have also improved, through smaller tags, denser labeling, and better compatibility with live-cell imaging [4,5]. Since its inception, a plethora of software packages have been written to perform the first step of the analysis: each SMLM image is in fact the estimated spatial distribution of thousands or millions of single molecules, imaged, identified and localized individually over the course of a few thousand frames. The identification and precise localization of each molecule affects the quality of the image: the higher the density of successfully identified molecules, combined with a lower uncertainty of each molecule's position results in an increased resolution of the final image. While the first aspect can be addressed primarily by improving sample preparation and image acquisition, the latter also relies on the ability of the software to perform localization. Given the importance of the software in SMLM and the amount of available options, two separate challenges [6,7] were organized within the framework of the ISBI Grand Challenges, where participants from around the world were invited to apply their own software to a set of defined, realistic, simulated datasets, where the ground truth (i.e. the position of each individual molecule) was kept hidden. Compared to a single group performing comparisons across all software packages, this format has the advantage of giving each software author or expert user the chance to get the best possible result from the software. Both challenges are now running challenges, accepting submissions on a continuous basis and the current leaderboard can be explored at http://bigwww.epfl.ch/smlm/challenge2016/leaderboard.html. This work will present an overview of the current state-of-the-art in SMLM analysis, the rationale behind the competitions, and some of the remaining challenges, including the question of whether the quest for defining resolution in SMLM is over.

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