Combining Convolutional Neural Networks and Maximum Likelihood Fitting for Robust High-Density Single-Molecule Localization

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Key to single-molecule localization microscopy (SMLM) is the sparse activation and localization of single fluorphores. Maximum-likelihood fitting using experimentally derived point-spread-function (PSF) models can reach the theoretical minimum uncertainty in all three dimensions [1].
Increasing the density of activated fluorophores beyond the single-molecule regime would greatly speed up this intrinsically slow technique, facilitating live-cell and high-throughput imaging. In addition, this also enables the use of fluorphores with sub-optimal blinking characteristics. Unfortunately, multi-kernel fitting of overlapping PSFs is not robust for 3D samples, and accurate starting parameters are required to avoid getting trapped in local minima.
In contrast to multi-kernel fitting, machine learning algorithms utilising convolutional neural networks (CNN) have been shown to lead to robust position estimates of overlapping emitters, even in 3D [2]. However, the accuracy depends strongly on the training and might not reach the theoretical limit.
Here we combine the best of both worlds: we use a CNN as a robust initialisation and model selector and feed this information into a high-density maximum-likelihood fitting algorithm, which in principle can achieve optimal precision. This combination has the prospect of fast, robust and accurate high-density localization in 3D.

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