Publisher Correction: Molecular motion on ice

Amber Dance

Correction to: *Nature Methods* https://doi.org/10.1038/s41592-020-0940-7, published online 19 August 2020.

In the version of this article initially published, the first paragraph of section "The need for speed" referred to transcription termination. It should have referred to translation termination. The error has been corrected in the PDF and HTML versions of the article.

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Addendum: Parameter-free image resolution estimation based on decorrelation analysis

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Recently we reported a new method for the estimation of the resolution of light microscopy images. Our goal was to demonstrate that that method is applicable to any kind of microscopy images, including single-molecule localization microscopy (SMLM). Unlike other super-resolution methods, single-molecule localization datasets must be rendered in order to be visualized. Ideally, the resolution estimate should not depend on the method chosen to render the data. In practice, however, this is not the case and commonly used rendering methods implicitly make additional assumptions about the underlying localization statistics, which in turn can have an impact on the estimated resolution obtained using decorrelation analysis.

In our publication, we rendered all the localizations as a Gaussian with a standard deviation equal to their respective localization uncertainty. We acknowledge that the results we presented may not apply if another rendering method is used. Here we discuss the impact on our resolution estimation method of three standard approaches for SMLM dataset rendering: histogram rendering, fixed Gaussian rendering and localization-uncertainty-based Gaussian rendering.

Localization-uncertainty-based Gaussian rendering

Localization-uncertainty-based Gaussian rendering is the method we used in the original publication. We have shown, using simulations and experimental data (see Fig. 4 and Supplementary Results 7 of the original article) that this was a valid rendering choice for localization datasets and that we were able to get a meaningful resolution estimate. However, the estimation of the localization uncertainty itself is not a trivial task and depends on several parameters—for example, a proper camera calibration to estimate photon counts. Ideally, the resolution estimation should not depend on external parameters and should be contained in the localizations themselves. Hence, we discuss the impact of two alternative standard rendering methods on our resolution estimation method that only consider the localizations' locations: fixed Gaussian and histogram rendering.

Fixed Gaussian rendering

Fixed Gaussian rendering consists in displaying each localization as a Gaussian with a small fixed standard deviation. While being a fast and perfectly valid choice in terms of visualization, this method is implicitly making a strong assumption concerning the data. If we could experimentally repeat the localization procedure of a single emitter an infinite number of times and render the data as a histogram, the result would approximate a Gaussian-like distribution with a spatial spread equal to the localization precision, centered around the true position of the fluorophore. In contrast, when we use a Gaussian to render a single localization event, it will be centered around the measured position. We thus make the strong assumption that the measured position is the true position of the fluorophore and that the localizations are spread according to the chosen standard deviation. This assumption is also made by the localization-uncertainty-based Gaussian rendering, albeit with a physically meaningful localization spread.

Let us consider an SMLM dataset of N localizations with positions $\boldsymbol{p}_n = [x_n, y_n]$. The rendered image using a fixed Gaussian as a kernel is, in Fourier space:

$$I_G(\boldsymbol{k}, \sigma) = \sum_n^N e^{i\boldsymbol{k}\boldsymbol{p}_n} G(\boldsymbol{k}, \sigma) = F(\boldsymbol{k}) G(\boldsymbol{k}, \sigma)$$

where $\boldsymbol{k}$ is the reciprocal space of $\boldsymbol{p}$, $F(k) = \sum_n^N e^{i\boldsymbol{k}\boldsymbol{p}_n}$ is the Fourier transform of the Dirac distribution and $G(\boldsymbol{k}, \sigma)$ is the Fourier transform of a Gaussian distribution of standard deviation σ. The object spectrum is simply weighted by a Gaussian. From an image-processing point of view, this corresponds to a filtering of Fourier space and is a direct manipulation of the frequency content of the image. The estimated resolution of $I_G(\boldsymbol{k}, \sigma)$ is therefore a function of both the object spectrum $F(\boldsymbol{k})$ and the (arbitrary) Gaussian filter size σ. If the