Method for determining localization precision

[Signal-to-noise ratio]

For each of the cropped image, the signal intensity recorded for a single molecule is defined as the average pixel intensity of the center block (total 9 pixels) subtracted from the average of the border (depending on the cropped image size, assuming it is large enough to represent the background intensity). Since we determine the localization precision of the experiment from the diffraction limited spot of a single molecule, we estimate the noise within this diffraction-limited spot as the square root of the signal intensity. Using the average raw intensities from the experiments, we calculate a signal-to-noise ratio (SNr) of ~17.

*\* see snr.tif and the corresponding example\_video\_1.tif*

[Particle localization]

We determine the sub-pixel position of each molecule from CCD images using a radial-symmetry-based localization method (Ref-1). The method calculates the intensity gradient at all points of an image and analytically determines the point of minimal distance to lines parallel to these gradients, which corresponds to the symmetry center. We use the symmetry center as the tracer position for diffusion analysis.

[Accuracy]

The localization accuracy of our analysis is determined by simulating CCD images of tracers centered randomly positioned in the range of ±0.5 pixels and then we compare known and estimated positions at different signal-to-noise ratios. Simulated tracer images are created as in ref-1. In brief, tracers are simulated as a point source at some position and convolved with a theoretical point spread function, after which the resulting image is pixelated at the same scale as the experimental images and subjected to Poisson-distributed noise with some desired signal-to-noise ratio, as defined in former paragraph. This yields a localization precision of ~ 9 nm.

*\* see benchmark.tif*

Table 1. Simulated SNr’s. Each SNr corresponds to each increasing example image indices, reading from the table as left to right and up to down. It can be seen that the example-video has SNr similar to that of example-fig-9, which is SNr=16.2.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 7.71 | 7.87 | 8.21 | 8.88 | 9.79 | 10.9 | 12.4 | 14.1 | 16.2 | 18.6 |
| 21.5 | 24.8 | 28.6 | 33.1 | 38.2 | 44.2 | 51.1 | 59.1 | 68.4 | 79.1 |

Ref-1: Parthasarathy, R. Rapid, accurate particle tracking by calculation of radial symmetry centers. *Nature Methods* **9,** 724–726 (2012).