nature research

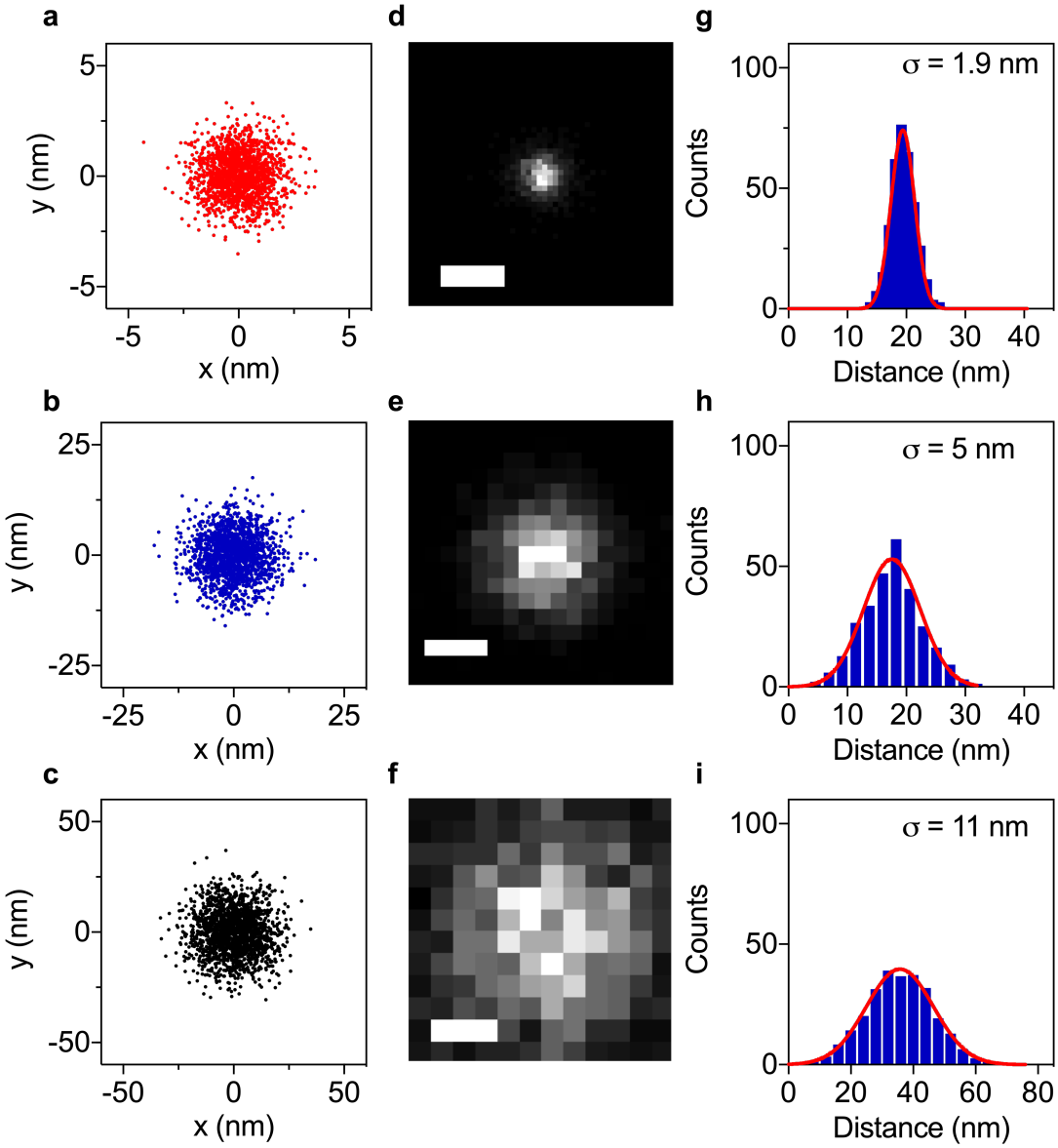
https://doi.org/10.1038/s41596-020-00426-9

Supplementary information

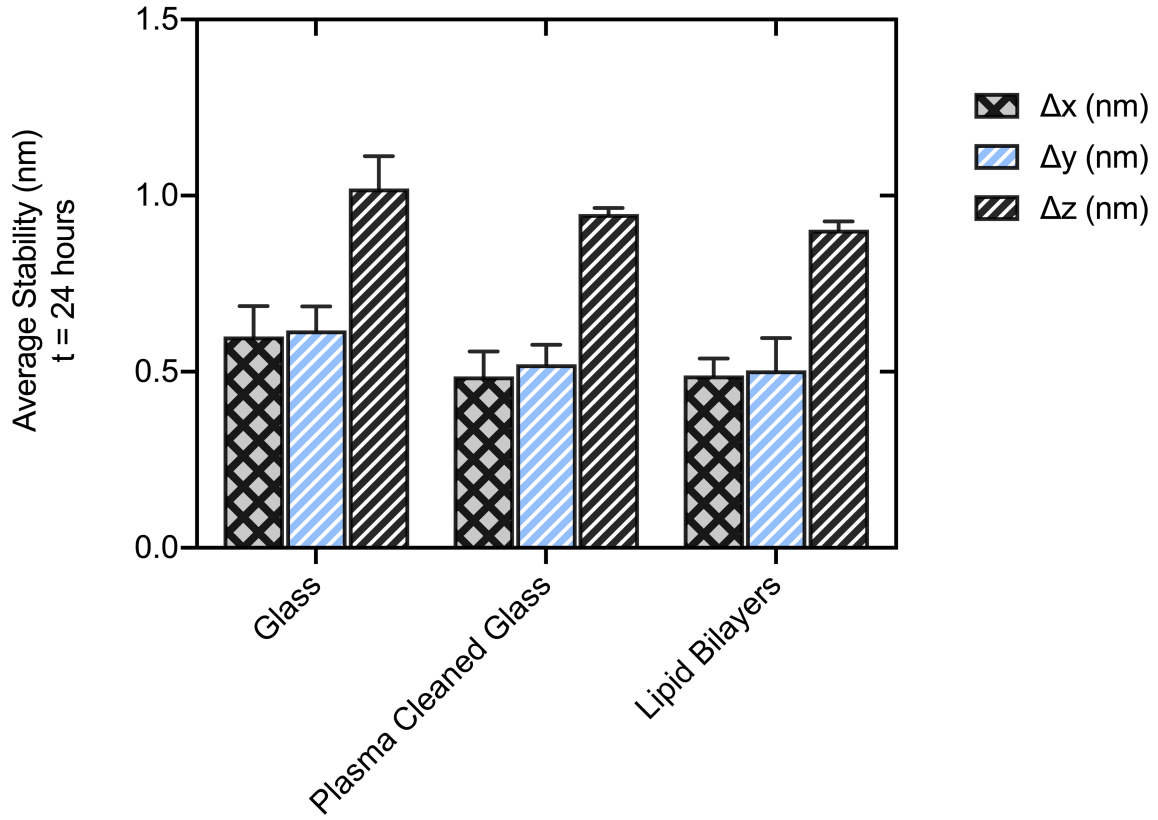
3D active stabilization for single-molecule imaging

In the format provided by the authors and unedited

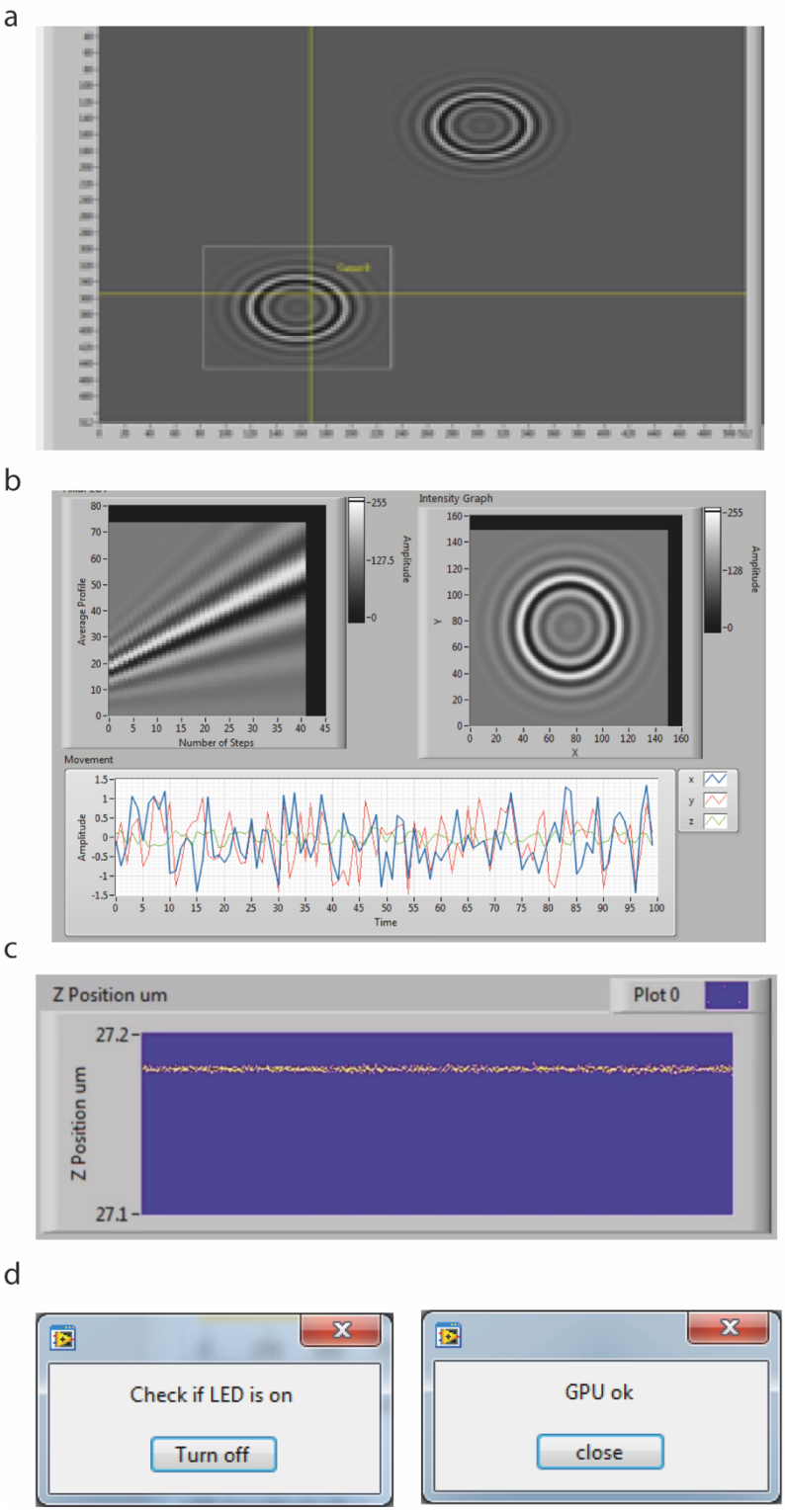
SUPPLEMENTARY INFORMATION



Supplementary Figure 1. Degradation of localization precision as a function of drift. **a-c**, Drift during acquisition was simulated by generating Gaussian noise with multiple standard deviations (SD) (N Points = 2000). Simulated drift of 1 nm in (**a**), 5 nm in (**b**) and 10 nm in (**c**). **d-f**, Simulated single molecule data with 1.5 nm localization precision was corrupted by the simulated drift. Scale bar = 10 nm. The magnification was adjusted to aid visualization. **g-i**, Fitted cross-sectional line profiles. Data can be found on GitHub online repository.



Supplementary Figure 2. Comparison of 3 different substrates on sample stability. Out-of-loop fiducials (n = 9) were monitored on **a**, clean glass; **b**, plasma-cleaned glass and **c**, lipid-bilayers. There is not a significant difference between the three surfaces after 24 hours. This indicates that the bulk of the BSA-biotin remains attached to the substrate; or that the biotin-streptavidin network in contact with the fiducial, operating as a whole, remains stable throughout. Data can be found on GitHub online repository.



Supplementary Figure 3. Overview of the simulation and hardware check software. **a-b**, Software simulation. **a**, Front panel of the simulation software used to generate and select fiducials. Fiducial selection is done by dragging a yellow crosshair above the bead. **b**, Simulation of axial LUT (left), movement is simulated in *xyz* (right) and the amplitude of the movement is then calculated and shown graphically (below). **c-e,** Hardware checks. **c**, Mad City Labs nanopositioner readout test. **d**, Infrared LED test. The LED turns on automatically. **e**, GPU test.