Supplementary Information (ESI) for "Characterizing Interaction of Multiple Nanocavity Confined Plasmids in Presence of Large DNA Model Nucleoid"

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1 Experimental setup

Our chip has a three layer stack structure (see supplementary Fig. 1a, d). The 100 nm silicon nitride membrane is interposed between the borofloat glass and the silicon frame. The loading channel and the nanofluidic cavities are etched into the borofloat glass (see supplementary Fig. 1b). The inlet and outlet of the loading channel are etched in the silicon frame by KOH etching (see supplementary Fig. 1a) We mechanically puncture the suspended membrane at the inlet and outlet manually right before the experiment. The membrane suspended above the trapping region functions as the flexible confinement lid and can be deformed via pneumatic actuation to vary the vertical degree of confinement. The inlet, outlet and control port are interfaced with a 3D printed chuck for buffer loading and introducing nitrogen pressure for pneumatic control. The chip is held on the chuck with a retaining ring to allow imaging (see supplementary Fig. 1c). The chip width is 10 mm (see supplementary Fig. 1e) A mounted chip ready for experiment is shown in supplementary Fig. 1f.

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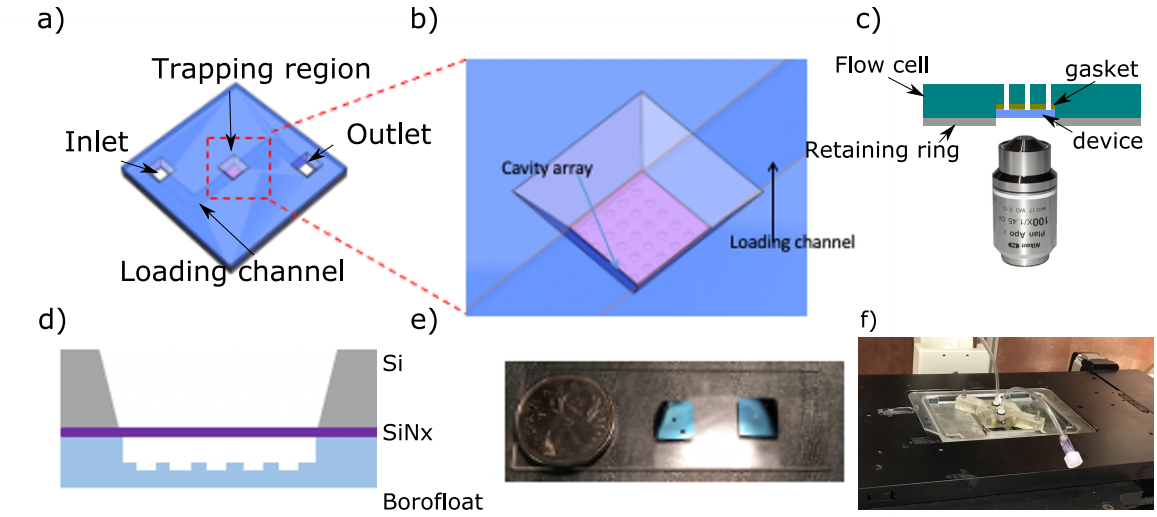
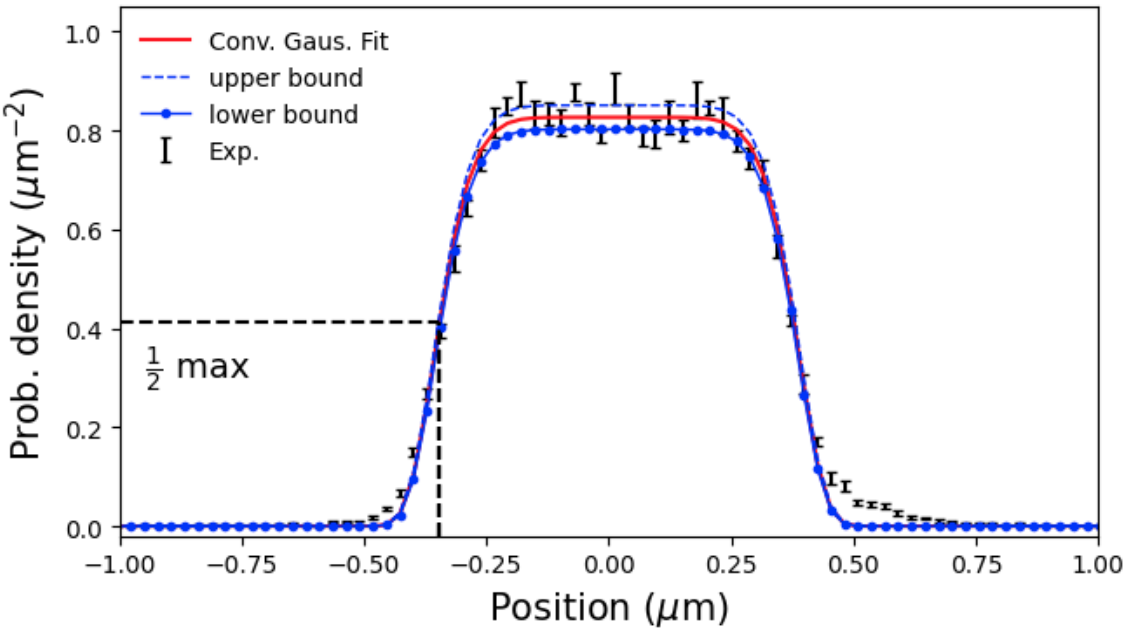


Supplementary Fig. 1: Schematic of the nanofluidic chip and the experimental setup. a) Chip schematic. b) Zoom-in schematic of the trapping region. c) The cross-section of the chip shown mounted to the 3D printed chuck. d) Cross-section showing cavities and trapping region. e) A photo comparing our chip to a Canadian quarter. f) Schematic showing a mounted chip on our microscope ready for experiments.

2 Plasmid Distribution Edge Extraction

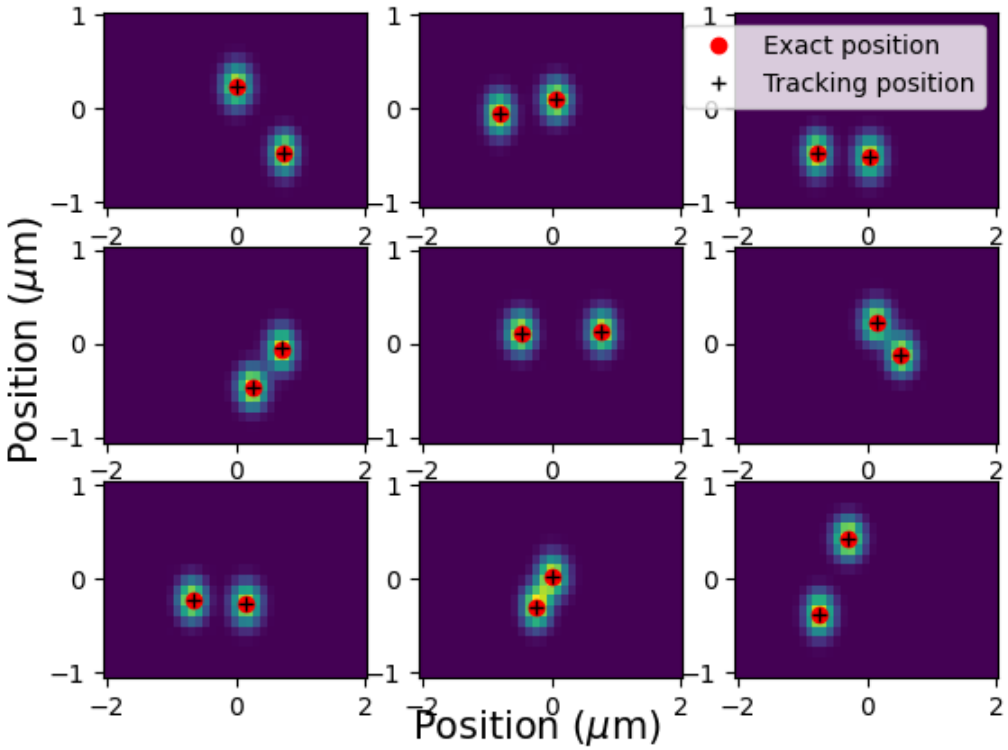
We extract the distance between the plasmid distribution edge and the wall by fitting the cross-section of the plasmid histograms with a broadened box function based on the convolution of a Gaussian with a rectangular step function. Here we show a typical fitting result from the single plasmid confined in the cavity with cap $e = 0.75$. The height, width and position of the square function and the standard deviation of the gaussian function are extracted from the least square fitting. We define the position of the distribution edge as the point where the plasmid CM probability density falls to one half of its peak value.



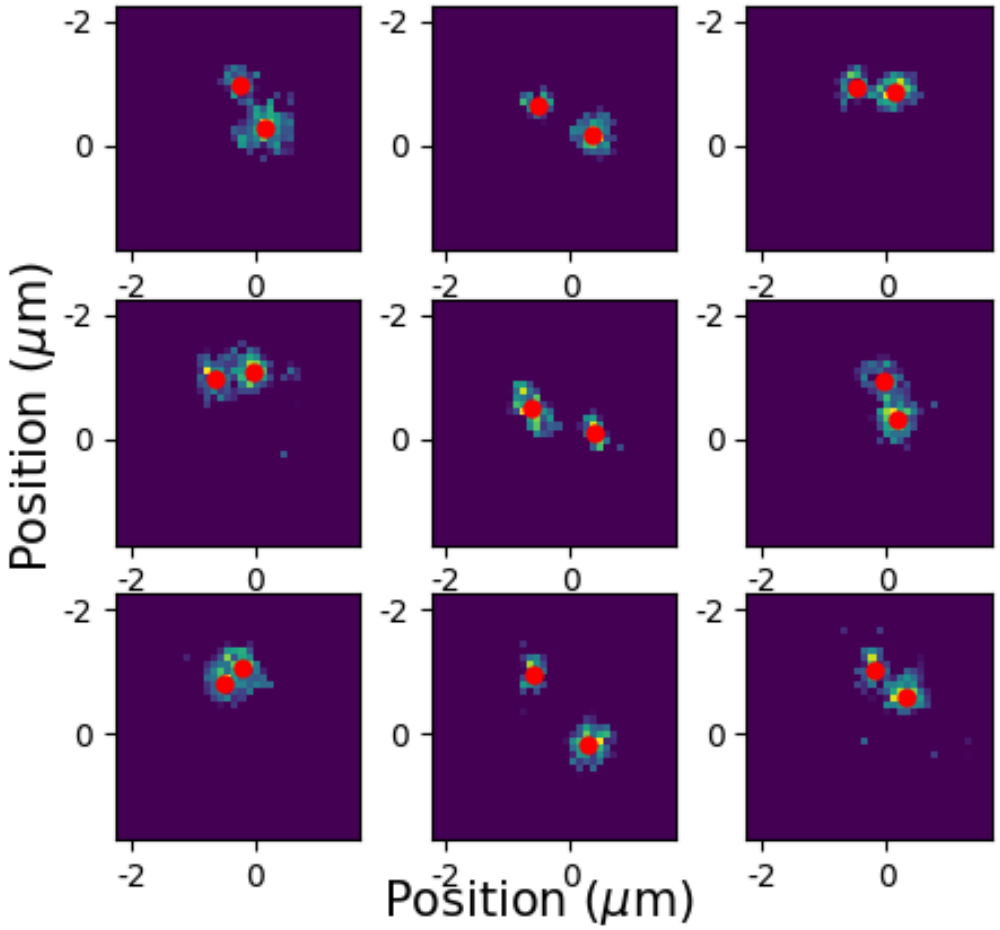
Supplementary Fig. 2: Extracting edge position from fitting to the plasmid histogram cross-section. The red line is the fitted convoluted gaussian box function. The data points indicate the mean value of the plasmid histogram within the 300 nm average window from the central cross-section and the error bars give the standard error of the mean over the same window. The blue dashed line and blue dotted line are fitted using the greatest and the smallest histogram value within the average window to give the error bars in Fig. 5 in the main context.

3 K-Means Clustering Based Multi-particle Tracking

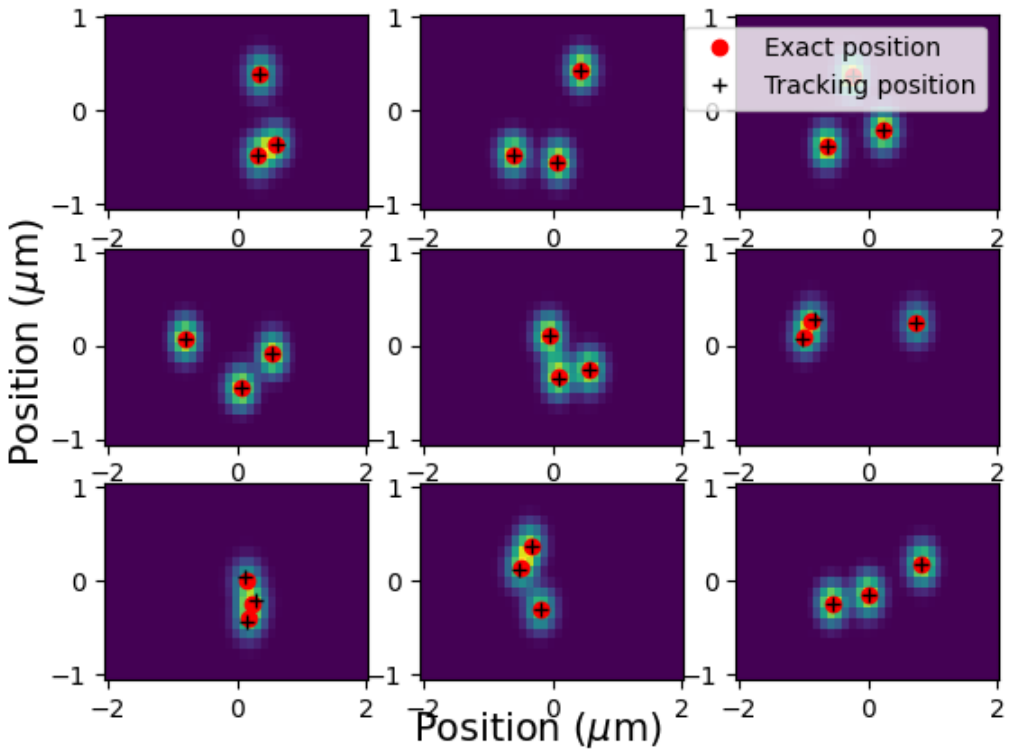
We validate the accuracy of our k-means clustering based tracking method by creating artificial plasmid images. To do this, we place plasmids randomly within the frame size and note down the actual position of each plasmid. We then convolve the plasmid position with the point spread function to construct a simulated image. We feed this simulated image to our K-means based tracker. Finally, we compare the actual position with the tracked position visually and calculate the tracking error by averaging the misplacement over 100k frames. The average error for the two plasmid tracking is 0.05 μm and the average error for the three plasmid tracking is 0.1 μm. In both case, the error is below the camera pixel size (0.11 μm).



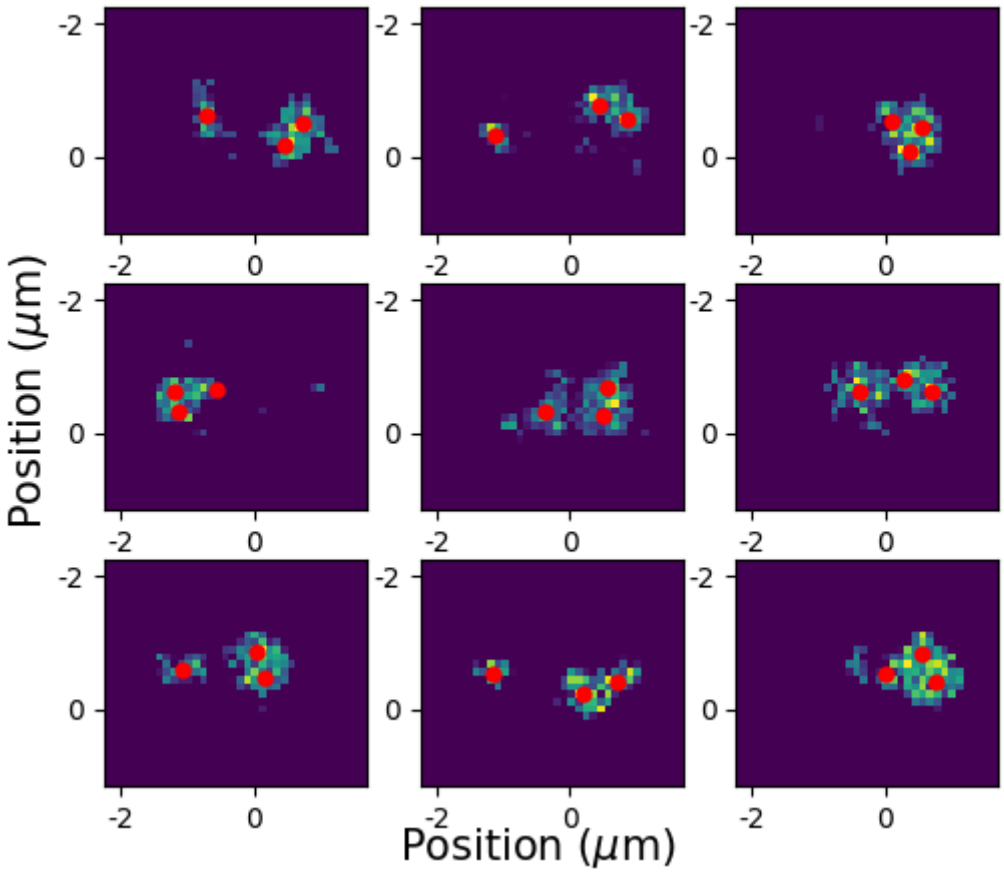
Supplementary Fig. 3: K-means clustering based tracking for simulated two plasmids images. The red dots are the actual position of the plasmids. The crosses mark the tracked position.



Supplementary Fig. 4: K-means clustering based tracking for experimental two plasmids images. The red dots are the tracked positions.



Supplementary Fig. 5: K-means clustering based tracking for simulated three plasmids images. The red dots are the actual position of the plasmids. The crosses mark the tracked position.



Supplementary Fig. 6: K-means clustering based tracking for experimental three plasmids images. The red dots are the tracked positions.