Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information for Automated Electrokinetic Stretcher for Manipulating Nanomaterials

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Trapping of 63 nm bead

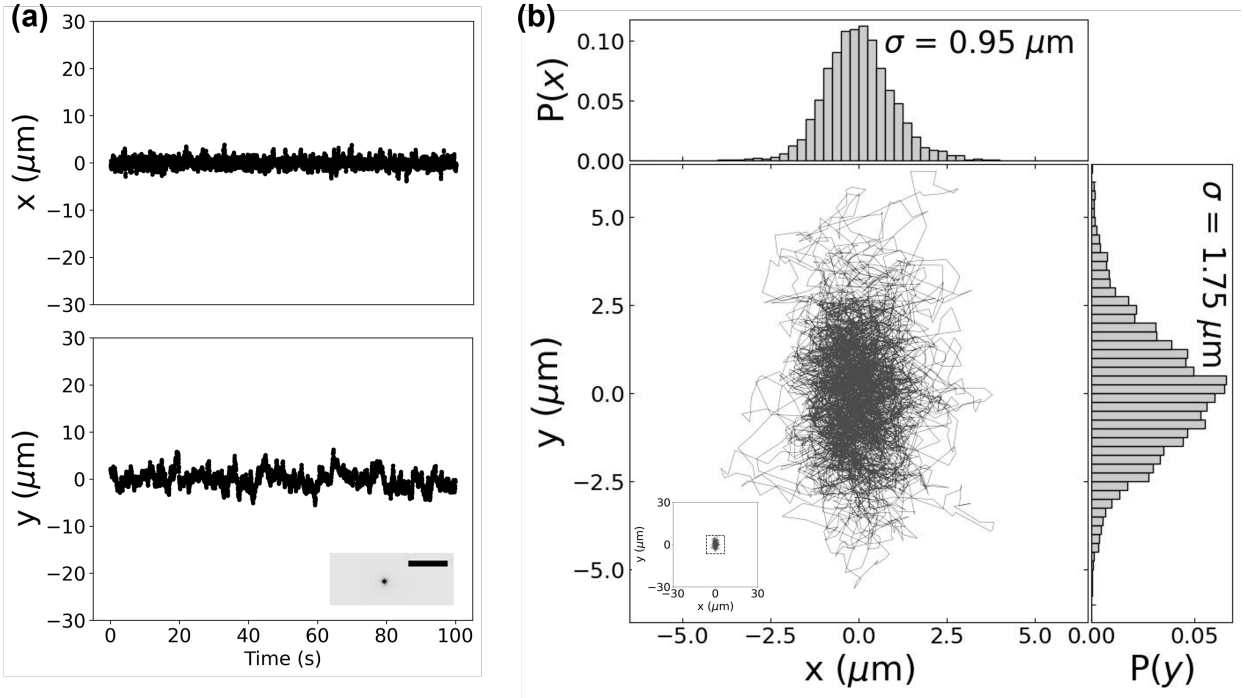

Figure S1: Trapping of 63 nm bead. Trapping of a single 63 nm polystyrene bead using a 200 V stretching voltage, proportional gain setting of 16.7 V/μm and a frame time of 20 ms. (a) Trajectory of the 63 nm bead along the elongational (x) and compressional (y) axes with time. Inset: Image of trapped bead. For clarity, the image's intensity gradient has been inverted. Scale bar represents 5 μm. (b) Trajectory of the 63 nm bead in the elongational (x) and compressional (y) axes, with histogram of displacements of the trapped object from the trap center along each axis. Inset: Zoom out view of trajectories in the elongational (x) and compressional (y) axes.

Trapping of 190 nm bead at different voltages and gain settings

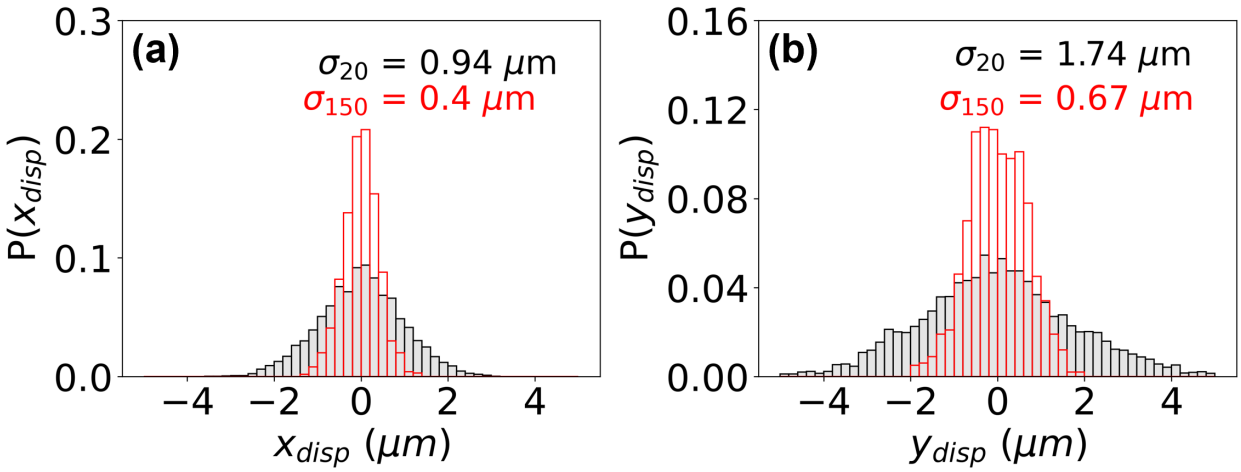

Figure S2: Trapping of a 190 nm bead at different voltages and proportional gain settings. Histogram of displacements of a trapped 190 nm polystyrene bead in the (a) elongational x and (b) compressional y axes using a 20 V stretching voltage and proportional gain setting of 1.1 V/μm (black), compared to a 150 V stretching voltage and proportional gain setting of 13.3 V/μm (red). The bead trapped at 150 V and with a gain setting of 13.3 V/μm shows much tighter particle confinement.

Linear fitting parameters for Figure 4c

$\gamma_{eff} = -2.56 \times \text{Gain} + 31.3$

R^2 value = 0.880

Flowchart for high-throughput stretching algorithm

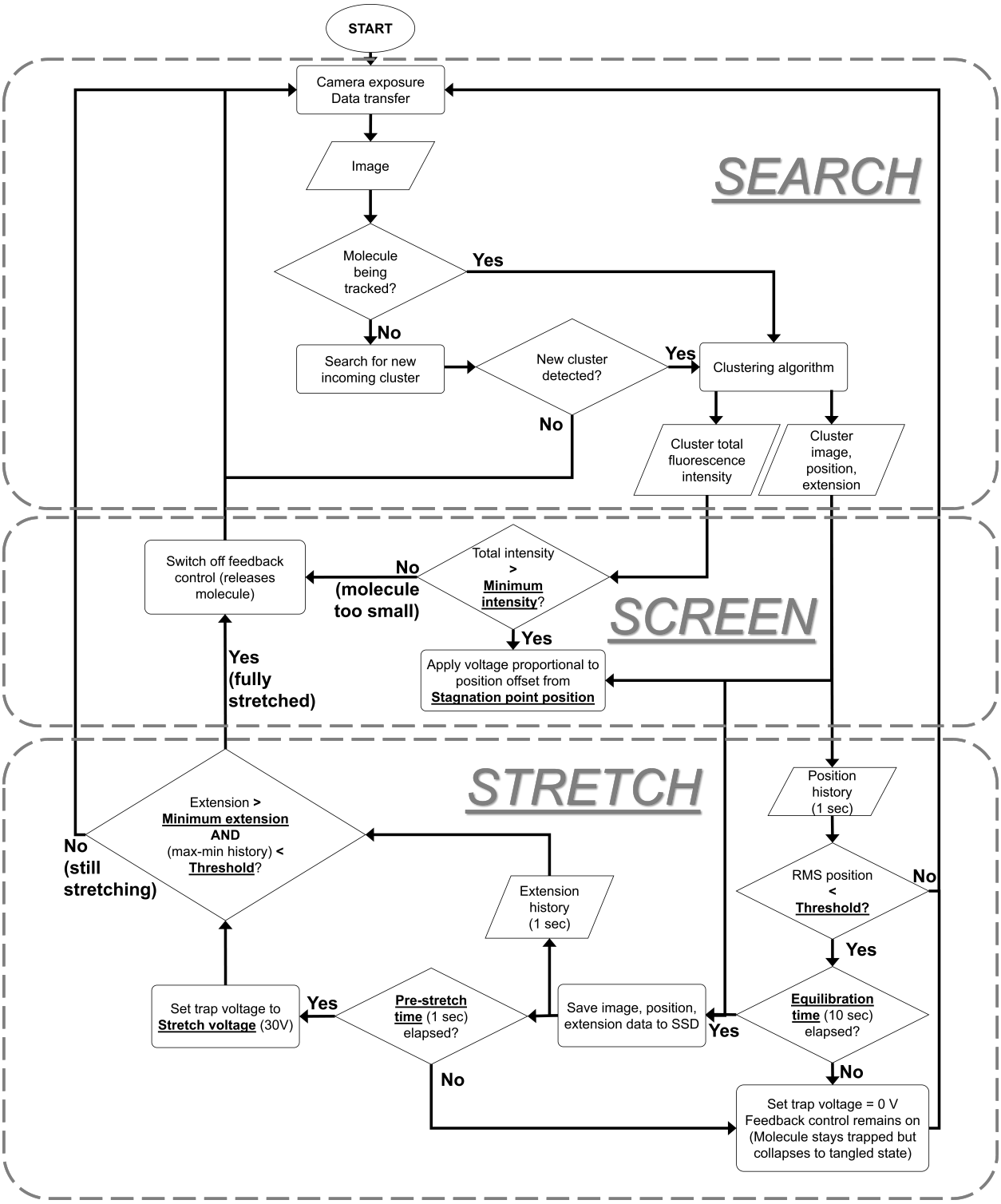

Figure S3: Flowchart summary of the automated DNA stretching program.

Supplementary Video S1: Automated, high-throughput stretching of DNA molecules

In Supplementary Video S1, we demonstrate the custom LabVIEW program executing automated, high-throughput stretching of DNA molecules. The program involves three phases – search, screen and stretch. As molecules flow through the channel under the elongational field, the program searches for a molecule to track and checks the fluorescence intensity of the molecule. If the fluorescence intensity of a selected molecule is below a pre-determined threshold (top left panel), the program releases the molecule and searches for a new one. Once the program determines that a molecule is fully intact, the molecule is brought to the stagnation point. The voltages (top right panel) are then turned off and the molecule is allowed to equilibrate at 0 V. After a given amount of time, the program begins to record a video. The view of the full ROI (bottom left) is turned off to minimize system latency and only a smaller ROI (bottom right) is available for user observation purposes. The voltages are turned on to generate a planar elongational field and the molecule stretches. When the program has determined the molecule to have reached full extension, video recording is turned off and the full ROI is available again. The process is then repeated to collect hundreds of video recordings within hours.

Further discussion on automated platform throughput

Microfluidic systems for studying polymer dynamics at the single-molecule level are typically low-throughput. In addition, performing single-molecule manipulation generally requires extensive training and skill to obtain reproducible measurements. For stagnation point trapping, the exact throughput is dependent on the skill and experience of the experimentalist in trapping and actively maintaining a molecule at the stagnation point while it stretches. For a dexterous user with years of experience performing such experiments, we estimate a maximum throughput of 1 molecule every 2-3 minutes. With an automated system, the rate-limiting step shifts to the time it takes for a molecule to stretch – i.e. the actual experiment – rather than having the researcher's technique as a bottleneck. For the given protocol, the automated system guarantees a throughput of 1-2 molecules per minute. Furthermore, because the experiment is self-driving, researcher time is freed up to perform other tasks, such as real-time data analysis of the dataset under collection or analysis on a previously obtained dataset. To visualize this time-efficiency advantage, we compare the estimated hours involved in collecting a dataset of 100 DNA molecules stretching in a planar elongational field using the automated and manual systems (Fig. S4). Due to the lower throughput of manually performed experiments, it is likely that multiple experiments have to be run over several days to complete the data collection. While data analysis can be performed alongside data collection for the automated system, it has to be done sequentially for the manual system due to the availability of the experimentalist. Overall, using the automated system results in at least a two- to threefold increase in experimental throughput.

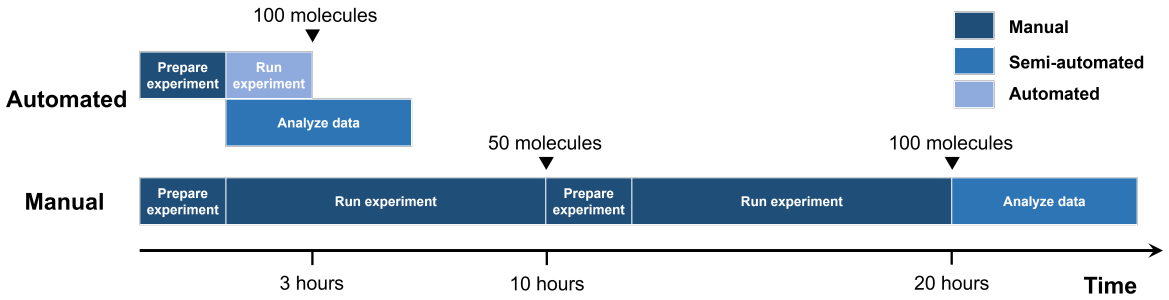

Figure S4: Comparison of estimated hours needed to collect a dataset of 100 DNA molecules stretching in a planar elongational field using an automated versus manual system. For a manual system, a typical data pipline consisting of sample preparation, experimentation and analysis is performed sequentially, with the experiment time subject to researcher skill and experience; with an automated system, researcher time is freed up for parallel tasks. The data analysis in both cases can be considered semi-automated, as it will generally combine existing code, development of new code and visual verification of collected data and analytical results

Size-based sorting of DNA molecules

Apart from trapping and stretching objects for long observation times, the electrokinetic stretcher presented in this work can also enable other types of contact-free manipulation of micro- and nanoscale objects. For example, the stretcher can be used to perform size-based sorting of molecules. This is demonstrated using a mixture of 48.5 kbp and 165.6 kbp DNA molecules, as illustrated in Fig. S5. In the example presented, both long and short molecules are captured around $x = 25$ μm from the center of the trap, before being brought to the stagnation point. The molecules remain trapped at $x \sim 0$ μm while they are stretched and classified as long or short based on their maximum projected length along the elongational axis (40 μm and 10 μm for the long and short DNA, respectively). After classification, the trapping voltage is turned off and the molecules return to a coiled configuration. The molecules are then moved towards either the left or right reservoir ($x \sim -30$ μm and $x \sim 40$ μm, respectively). Finally, the system releases the classified molecule and awaits a new molecule to capture and sort. The capability to sort nanoscale objects is highly sought after in the field of nanomaterials, and we can envision our platform as a high-throughput sorter for nanoscale or microscale objects. We highlight that the flexibility of the platform opens up a multitude of possibilities for downstream applications. For example, while we have demonstrated sorting of DNA molecules based on size, it is equally feasible to sort objects based on other observable features, such as shape or fluorescence intensity.

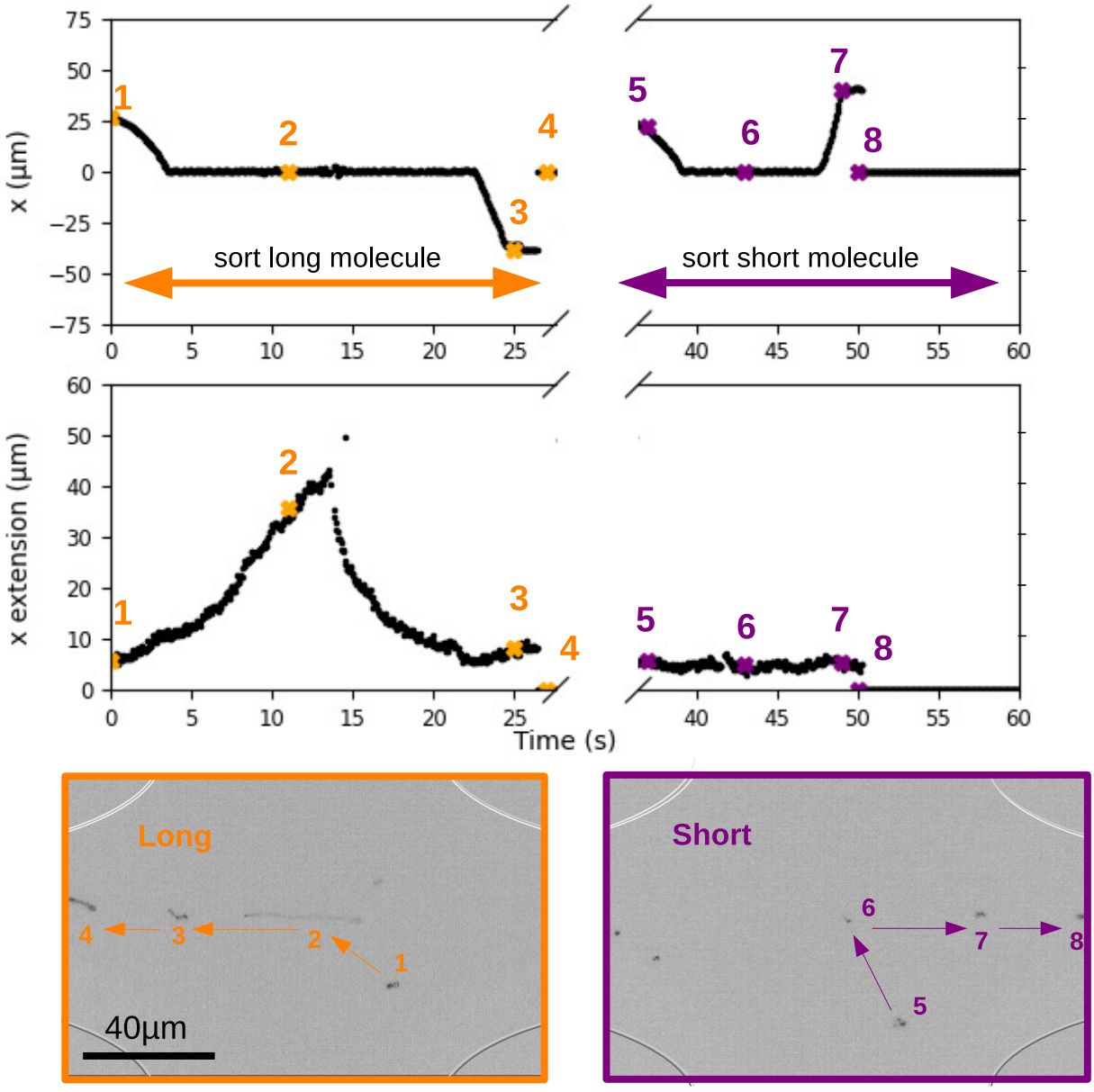

Figure S5: Sequential trapping and sorting of two DNA molecules. Top: Location along the x axis of a long (orange) and a short (purple) DNA molecule. Middle: Variation in extension length of the DNA molecules throughout the sorting process. Bottom: Overlay images showing the displacement and morphology of the two molecules in the center of the microfluidic channel. For clarity, the images' intensity gradients have been inverted.