SUPPLEMENTARY MATERIAL

1. DNA END-FUNCTIONALIZATION

End-functionalized DNA is prepared using linear bacteriophage λ-DNA (New England Biolabs, Beverly, MA) with sequence-specific 12 base polynucleotide overhangs; 3’-end overhang is gggcggcgaccg, and 5’-end overhang is aggtcgccgccc. Complimentary biotin and digoxigenin tagged oligomer sequences (Sigma-Aldrich, St. Louis, MO) are ligated to λ-DNA using T4 DNA ligase (New England Biolabs) following standard protocols (1, 2). The DNA molecules are thus able to bind to streptavidin-coated superparamagnetic beads on the 3’ end, and antidigoxigenin-coated beads on the 5’ end.

2. BEAD PREP

The anti-digoxigenin beads are prepared by first rinsing 3 μm beads (Polysciences, Warrington, PA) with 1X phosphate buffered saline (PBS) (10010-PBS, Life Technologies, Frederick, MD). After centrifugation, beads were re-suspended in 5% gluteraldehyde 1X PBS and incubated overnight at 4° C. Then the beads were rinsed and re-suspended in 0.2 mg/mL anti-digoxigenin (Roche, Indianapolis, IN) in 1X PBS for 4 hours. Subsequently, the beads are centrifuged and re-suspended in 0.5 M ethanolamine (Acros/Thermo Fisher Scientific, Waltham, MA) and 1X PBS for 30 minutes at room temperature. After centrifugation, the beads are re-suspended in 10 mg/mL BSA (Sigma-Aldrich) in 1X PBS and incubated for 30 minutes at room temperature. After repeating the last step twice, the polystyrene beads are ready to bind to the end-functionalized 5’ end of DNA. The M-280 streptavidin-conjugated superparamagnetic beads are purchased from Dynal Biotech (Oslo, Norway). They have saturation magnetization of 10 Am^2/kg (mass) and 14 kA/m (volume) and remain saturated 2500 μm from the magnet.
The PBS formulation is KH2PO4 (1.0588 mM), NaCl (155.172 mM), and Na2H PO4 - 7H2O (2.9664 mM).

3. DNA-BEAD TETHERING

The end-labeled DNA was then incubated in 330 μL Tris-EDTA (Sigma-Aldrich) with streptavidin-coated superparamagnetic and anti-digoxigenin-coated polystyrene beads. Incubation was carried out at 28º C to 30º C overnight in an incubator for at least 8 hours but we found that 14 hours yields more tethered DNAs. Tris-EDTA (TE buffer) consists of 10 mM Tris-(hydroxymethyl)-aminomethane adjusted to pH 8.0 with HCl and 1 mM EDTA (diaminoethane tetraacetic acid).

4. WIF-B CELL CULTURE

WIF-B cells were grown in a humidified 7% CO2 incubator at 37º C as described (3). Briefly, the cells were grown in F12 medium (Coon’s modification) (Sigma-Aldrich), pH 7.0, supplemented with 5% fetal bovine serum (Gemini Bio-Products, West Sacramento, CA), 10 μM hypoxanthine, 40 nM aminoterpin and 1.6 μM thymidine. Cells were seeded onto 10 cm dishes at 1.3 × 10⁴ cells/cm² and cultured for 8 - 12 days until they reached maximal density and polarity.

5. HISTONE PURIFICATION & ANALYSIS

Confluent monolayers of WIF-B cells were lysed in 0.8 ml of extraction buffer and histones purified using the spin-column-based Histone Purification Mini Kit (Active Motif, Carlsbad, CA) according to the manufacturer’s instructions. Preparative fractions and final eluates were
mixed with Laemmli sample buffer (4) and boiled for 3 min. Proteins were electrophoretically separated using SDS-PAGE and visualized by staining with Coomassie Brilliant Blue. Purified histone concentrations (~ 1 mg/mL for each experiment) were determined by densitometric analysis of Coomassie Brilliant Blue stained gels using bovine serum albumin as the standard. The purified histones were aliquoted and stored at -80°C. Immediately before use, the histones were diluted to 0.167 mg/mL in 1X PBS.

6. MICROPIPETTE PULLING & POLISHING

The aspiration micropipettes are formed by drawing 1mm, thick-walled, glass capillaries (World Precision Instruments, Sarasota, FL) in a horizontal micropipette puller (Sutter Instruments model P-97, Novato, CA) and then polishing in a microforge resulting in stiff micropipettes with 1 - 2.5 μm openings (estimated by comparison to the diameter of aspirated polystyrene beads.) The following settings are used in the P-97: heat = 589, pull = 25, velocity = 110, and time = 140. The protein injection pipette is formed using a vertical micropipette puller (Narashige PP-83, Narashige USA, East Meadow, NY) with default settings that slowly draws out a ~2.5 cm-long taper on the standard glass capillaries described above. The resulting pipette has to be broken or cracked at a position along its length so that two 10 μm – 20 μm inner diameter tapered pipettes are formed. The location where the pulled pipette must be broken so as to have a tip with ~15 μm inner diameter is determined by trial and error. A small piece of glass capillary or a glass cutting tile is used to make the break. There is no need to further polish this pipette using the microforge.

7. PIPETTE CLAMP, SAMPLE CELL HOLDER, & RAIL SYSTEM FOR MOUNTING
A 35 mm × 25 mm × 12 mm thick custom-designed, two piece, all-Aluminum adapter is used to clamp the aspiration and protein microspray pipettes to the hydraulic, three-axis micromanipulator and was manufactured in our machine shop. Figure S1 shows its design. Two 1.5 mm wide diagonal channels making an angle of 12° with each other are cut into the top segment. The micropipettes are placed in the grooves and the bottom segment is bolted with the top segment to clamp the micropipettes into place.

The sample cell holder consists of two parts: a custom horizontal piece designed to carry the sample cell, and a vertical adapter that bolts to the electronic micromanipulator. The horizontal piece is machined from aluminum and has dimensions of roughly 100 mm × 80 mm × 10 mm. An opening of approximately 30 mm × 25 mm is created in the middle of the horizontal piece. The sample cell is affixed across this opening which allows the sample to be illuminated and imaged with the microscope. The vertical piece of the sample cell holder attaches to the end of the horizontal piece with 2 screws and also attaches to the electronic micromanipulator with 2 screws. The total mass of the two pieces is approximately 250 g.

The linear rail system (Thomson Linear, Radford, VA) consists of two rails and two carriages which enable coarse positioning of the (non-motorized) hydraulic micromanipulator with respect to the microscope objective. The rails are 15 mm size and 300 mm in length, while the carriages are standard Thomson Linear ball-bearing models intended for the 15 mm rails. The rails are bolted to the microscope side platform at a separation of 6 inches; the rail carriages are connected via a custom machined horizontal plate of aluminum. The hydraulic micromanipulator
is positioned on this horizontal plate using a custom machined mounting block. Generally, once the micropipette is loaded onto the hydraulic micromanipulator and located in the microscope objective, no further adjustments in the position of the micropipette are made during the experiment.

8. MAGNETIC TWEEZERS

Fig. S2 shows components of the assembled tweezers, which are labelled by color-coded lines bordering each item. Not shown are the CCD sensor, syringe pumps, zoom lens, and illumination source. These are described in the manuscript and associated figures.

We would like to note some points regarding the objective’s limited \( z \)-resolution. The objective depth of field is approximately 900 nm, which means that relative to the aspirated (non-magnetic) bead, the magnetic bead can be 450 nm above or below the horizontal line through the non-magnetic bead and the geometric center of the magnet. This leads to a potential loss of extension due to projection effects of about 24 nm. However, the technique for sub-pixel localization of the optical centroids of beads using changes in the diffraction rings can also be applied to sub-voxel detection; this leads to better \( z \)-localization than nominally possible by the objective’s depth of field. We also note that the aspiration micropipette is first aligned with the center of the magnet and that point is used as the reference. When a tethered pair is found, the pipette is brought to height where the line from the center of the bead to the center of the magnet is parallel to the sample cell floor. Since the magnetic field geometry is constant, this means that the superparamagnetic particle should be constrained in a much smaller volume than implied by the depth of field. Further, DNA-protein studies involve measuring changes in extension. Since
any z-correction should be constant, the effect will cancel out when we compute the difference in extensions to obtain step lengths. The measured precision takes into account these effects and quantifies the variation in the force and extension measurements under near-identical conditions due to all sources. We quoted in Fig. 3C the precisions as determined by repeated experimental sampling, and so we conclude that the considerations described above lead to attenuation of projection effects.

Thermal fluctuations set the resolution limit for extension measurements. Additionally, video-microscopy-based centroid localization contributes to the final resolution. The thermal limit can be estimated using
\[ \delta x = \sqrt{\frac{4\beta k_B T \Delta f}{\alpha^2}} \]
as discussed in (7). Here, \( \alpha \) is the stiffness, \( \beta = 6\pi \eta a \) and is the drag coefficient, \( a \) is the bead radius, \( \eta \) is the viscosity of the buffer, \( \Delta f \) is the cut-off frequency, and \( K_B T = 4.1 \times 10^{-21} \text{J} \). At 10pN, \( \alpha = 2.66 \times 10^{-5} \text{N/m} \), \( a = 1.5 \times 10^{-6} \text{m} \), \( \eta = 8.9 \times 10^{-4} \text{Pa s} \), and \( \Delta f = 15 \text{Hz} \), leading to a thermal limit for extension resolution of 2.96 nm.

The contribution of the centroid localization scheme can be estimated using the expression given in (8):
\[ \frac{\delta x}{\Gamma} \approx \frac{1.8}{\text{SNR}} \sqrt{\frac{1}{N_{\Gamma}}} \]
Here, \( \delta x \) is the error in centroid localization, \( \Gamma \) is the half-width (or radius) of the image spot (or object of interest), SNR is the signal-to-noise ratio of the detector (the CCD camera in this case), and \( N_{\Gamma} \) is the number of samples in the distance \( \Gamma \). The SNR of the camera is 45.12 dB which gives a dimensionless SNR of \( 10^{\frac{45.12}{20}} = 180.3 \); \( \Gamma \approx 0.8 \mu \text{m} \); and \( N_{\Gamma} = 13.79 \). This leads to \( \delta x = 2.2 \) nm. Corrections for finite integration time and other camera parameters are necessary for experiments with extremely short tethers (less than 1000bp), small microspheres (less than
micron), and higher applied forces (9). For the system discussed here, however, the forces, tether length, and bead size are all outside the limits where such issues are relevant.

The DNA-tethered bead in a magnetic trap can be modelled as a bead connected to a spring in a thermal bath. Since Brownian motion of the solvent molecules produce random motions of the bead, the corresponding spring extensions, which determine the forces on the bead by Hooke’s law, are also subject to fluctuations, and cannot be determined beyond a particular resolution limit. The smallest measured force can be estimated using $\delta F = \sqrt{4\beta K_B T \Delta f}$ from (7.) Here again, $\beta = 6\pi \eta a$ and is the drag coefficient, $a$ is the bead radius, $\eta$ is the viscosity of the buffer, $\Delta f$ is the cut-off frequency, and $K_B T = 4.1 \times 10^{-21}$ J. With $a = 1.5 \times 10^{-6}$ m and $\eta = 8.9 \times 10^{-4}$ Pa s, and $\Delta f = 15$ Hz, we get $\delta F = 0.079$ pN.

9. EXTENSION EXPERIMENTS

Aspiration micropipette is formed as described in section 6. It is then connected to a buffer-filled syringe using 1mm-inner diameter Tygon tubing (Saint-Gobain, Akron, OH). The syringe is connected to New Era syringe pump (New Era Pump Systems model NE-1000, Farmingdale, NY) to produce aspiration. The syringe puller is drawn at a withdrawal rate of 5.5 mL/hr to 7.5 mL/hr; this results in minimal perturbation to the DNA.

The aspiration pipette is loaded with Tris-EDTA buffer using a spinal tap needle (Gertie Marx needle, GM25124, IMD Inc, Hunstville, UT). This is done by inserting the needle into the large-diameter open end of the micropipette and filling. Filling is done by firmly holding the micropipette while simultaneously applying pressure to the syringe and slowly withdrawing the
spinal tap needle. The pipette is then connected to the tygon tubing. Next, the other end of the

tubing is connected to the syringe, the entire assembly is taken to the instrument, and an adaptor

(see section 7) is used to fix the pipette to the (non-motorized) micromanipulator; the syringe is

also secured to the syringe pump. The pipette is then located in the microscope field of view. We
test for blockages through a visual inspection of the length of the micropipette and by applying
pressure to the syringe. If a droplet is seen at the tip, the pipette is usable.

Experiments are performed in 1X Tris-EDTA buffer at room temperature (~25° C or 297 K). A
typical force-extension experiment is performed as follows. First, we position the cell over the
objective and then locate the center of the magnet face that is pointing into the volume of the cell

as shown in Figure 1A of the manuscript. It is important to locate this point since it is along a

line passing through this point that the external force is simultaneously perpendicular to the

magnet and in the x-z plane. Next, the aspiration micropipette is positioned in the field of view
parallel to the long axis of the magnet with its opening along the line through the origin. During
the course of an experiment, we often check for mechanical drift by stepping back to the face of
the magnet to see if any unwanted movement has occurred. Tris-EDTA buffer and the bead-
DNA constructs are then introduced. The latter settle to the bottom of the cell very quickly. The
second cover-slip is placed on top of the glass cell resulting in a buffer-filled 500 μL – 800 μL,
25 mm x 20 mm x 1 mm, five sided cell. Surface tension prevents buffer leakage from the
exposed side, and buffer evaporation is negligible under typical sample illumination conditions,
and over a time scale of hours.
After identifying a bead pair at the bottom of the cell (a trial-and-error process since the two bead-types are visually identical), we move the micropipette tip behind the polystyrene bead and begin aspirating. The construct is then brought into position along the force line described above, and this alignment is checked by determining whether the bead-DNA construct is perpendicular to the aspiration pipette and both beads are simultaneously in the focal plane (which ensures that the force points in the z-direction only.) The coordinates of the tethered pair are recorded once it is in position. We then begin moving the magnet at a rate of either 0.320 μm/s or 1.6 μm/s.

10. HISTONE-DNA EXPERIMENTS

After drawing the aspiration and protein microinjection pipettes, each micropipette is filled with appropriate buffer and connected to a buffer-filled syringe using 1mm-inner diameter Tygon tubing (Saint-Gobain, Akron, OH). The syringes are mounted to separate New Era syringe pumps (New Era Pump Systems model NE-1000, Farmingdale, NY) for the aspiration and microinjection pipettes. The syringe pullers are drawn at a withdrawal rate between 5.5 mL/hr and 7.5 mL/hr chosen to minimally perturbing the DNA. Proteins are sprayed at a rate of 1.5 mL/hour.

Aspiration pipettes are filled as described in section 9. Using micro-loader pipette tips (Cat. No. 930001007, Eppendorf, Hamburg, Germany), we loaded the protein injection micropipette with 7.5 μL of histone solution followed by standard Tris-EDTA buffer. An air gap between the buffer and the protein solution in the micropipette prevented mixing and contamination of the histone solution.
Aspiration and protein injection micropipettes are loaded with buffer and proteins, mounted via clamps to their respective micromanipulators, and positioned above the objective inside the cell. Tris–EDTA is introduced to the cell and any air at the tips of either pipette is evacuated. A slight back pressure is then created in the aspiration tip to capture pairs and also in the protein pipette to prevent flooding the sample cell with proteins prior to an experiment. DNA-tethered beads are then pipetted in and the second cover-slip is placed on the cell. The next step is to capture a tethered bead pair and test its response to a force of 10 pN in order to verify that it is a single molecule of λ-DNA. If it shows the expected extension of 16.4 μm, it is brought to a distance of 2000 μm from the magnet (force, ~1 pN), and histones H2A, H2B, H3, and H4 are introduced. Protein binding is detected by the visible shortening of the tether. Once compaction occurs, we allow the condensed DNA to remain at the injection position for 1 minute and then begin to slowly (0.320 μm/s) decrease the distance between the magnet and the DNA tethered bead pair. This corresponds to a loading rate of ~0.008 pN/s from 2000 μm to ~ 1000 μm from the magnet. (The force increases by ~8 pN over 52 min.) Thus, the force can be considered constant for each 30 sec time segment and increases by ~0.25 pN between successive intervals.

Protein binding was detected by the visible shortening, and usually full compaction, of the tether. Once compaction occurred, we allowed the condensed DNA to remain at the low-force, protein injection position for 1 minute and then began to slowly (0.320 μm/s) decrease the distance between the magnet and the DNA tethered bead pair. This corresponded to a loading rate of ~0.008 pN/s from 2000 μm to ~ 1000 μm with the force increasing from ~0.8 pN to ~ 9 pN. (It took 52 min to travel 1000 μm so over 52 minutes we increased the force acting on the DNA-histone complex by ~8 pN.) The force was thus roughly constant for each 30sec time segment.
and increased by ~0.25 pN between successive intervals. Experiments were continued past 1000μm until the starting extension was recovered around 20 pN. A typical experiment lasted for ~2 hours.

11. CENTROID LOCALIZATION

Localization of the bead centroids is performed during post-processing analysis of the experimental movie captures. This procedure begins with the user cropping the field-of-view to restrict the analysis to the region of interest that contains the DNA-bead construct. The software then steps through each frame to locate the bead centroids. This is accomplished utilizing a circular-specific Hough transform (5) which identifies the first and second maxima of the beads' diffraction patterns. Through determination of the concentric diffraction rings, a common center point, and, therefore, the optical centroid, of the beads can be established by the software. The output of this process is a list of XY+time coordinates for all bead observations throughout the experiment.

12. FORCE AND EXTENSION CALCULATIONS

DNA extensions and forces are calculated offline using the input from the custom optical-centroid localization discussed above. Transverse fluctuations are used to compute forces via the Fluctuation-Dissipation relation (6): \( f_z = k_B T \langle z \rangle / \langle (\delta x)^2 \rangle \). Here, \( \langle (\delta x)^2 \rangle \) is the transverse fluctuation of the magnetic bead, \( f_z \), the pulling force, \( \langle z \rangle \), the average extension, and \( k_B T = 4.1 \) pN nm. Extensions are calculated by subtracting the positions of the optical centroids of the beads.
13. STEP FINDING

A step finding algorithm was developed and implemented in MATLAB for analyzing the extension data from DNA-histone experiments – see (1) for details.

14. PARTICLE TRACKING

TRACKING ALGORITHM

The algorithm is designed to track densely-clustered, indistinguishable objects moving at high speeds in a single direction, henceforth labeled (unless otherwise noted) the negative-$y$ direction. Objects may be spawned at high rates, and thus the number of objects frame-to-frame is not constant. They are also allowed to exit from the scene before traveling the entire field of view; this accounts for the case of superparamagnetic particles as some beads will move downwards (while moving toward the magnet) and fall out of focus before traveling the full length of the imaged scene. (This happens because the beads are not neutrally buoyant in the experimental buffer.) The object centroids in each frame are inputs and have been computed in a pre-processing step. Because the particles are indistinguishable, a given particle in frame $t$ can be linked up with multiple particles in frame $t+1$. The correct assignment is determined using two criteria which are discussed below.

The algorithm relies on a two-step computation involving a scoring function to generate a set of possible trajectories, one for each particle, and a backtracking method to further refine the proposed set. The scoring function for an object $p$ at time $1$ and an object $q$ at time $2$ is as follows:
\[ W_{p,1;q,2} = \text{sgn}(y_{p,1} - y_{q,2}) \sqrt{\left(\frac{(x_{p,1} - x_{q,2})^2 + (y_{p,1} - y_{q,2})^2}{\cos(\arctan(x_{p,1} - x_{q,2})/y_{p,1} - y_{q,2}))}\right)} - DB_y. \]

The *signum* function gives the score a positive value for movement in the direction of the force and a negative value for movement in the opposite direction. The numerator term is the Euclidean distance between the two observations. The denominator is the angle formed between the direction of the force and the vector that connects the two objects.

The term, \(DB_y\), is used to bias the scoring value towards movement in the direction of the force, penalizing observation pairs that do not show substantial movement in the direction of the force. It is calculated by utilizing the mean \(y\)-coordinate for all objects in a frame and finding its difference between successive frames. Since the location of a particle in the next frame has not yet been definitively assigned, different particle pairings are considered and the minimum of the difference in the mean \(y\)-coordinate between the two frames is chosen as the \(DB_y\) value. Thus, the \(DB_y\) term represents an approximation of the minimum distance we should expect an object to move between successive observations based on the gross behavior of all object movements.

Scores for two consecutive times are arranged in a matrix of values of size \(m \times n\), where \(m\) is the number of objects at time \(t_1\) and \(n\) is the number of objects at time \(t_2 = t_1 + \Delta t\). The columns are indexed by particles at time 2 while the rows are indexed by particles at time 1. If an object at time \(t_1\) has negative scores for all possible matches with objects at time \(t_2\) – this would involve looking along a row – the particle is taken to have exited the field-of-view. Similarly, scanning down a column, if all entries are negative, the object has just spawned or entered the field-of-view and the algorithm will assign it a new tag or ID. The likely trajectory links are determined
by seeking the smallest positive score between two observations beginning with the object at \( t_2 \)
that is closest to leaving the field-of-view in the direction of the force and finding which object at
the preceding time, \( t_1 \), has the smallest positive score. These two objects are then assigned to the
same trajectory. The algorithm then moves to the object at \( t_2 \) that is next closest to leaving the
field-of-view and finds which of the remaining objects at time \( t_1 \) have the smallest positive score.
This process is repeated for all objects at time \( t_2 \).

These trajectories are checked as follows. For each object, the back-tracking procedure assumes
that an object observed at \((x_1, y_1, t_1)\) and again at \((x_3, y_3, t_3)\) is likely to have coordinates at an
intermediate time \( t_2 \), \((x_2, y_2)\) close to the straight line connecting \((x_1, y_1)\) and \((x_3, y_3)\). This is
quantified by calculating the following:

\[
D_{1,2,3} = \left[ \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2} + \sqrt{(x_2 - x_3)^2 + (y_2 - y_3)^2} \right] 
- \sqrt{(x_1 - x_3)^2 + (y_1 - y_3)^2}.
\]

Large \( D \)-values represent an intermediate trajectory observation that departs widely from the
direct path that connects observations at times \( t_1 \) and \( t_3 \). Clearly, smaller \( D \)-values are preferred;
the limit of \( D = 0 \) represents three points that lie exactly on a line. The list of \( D \)-values is
assembled for each object and sorted using a modified greedy-exchange algorithm. To save on
computational cost, permutations are prioritized as follows. We compute the \( D \)-values for the
scoring-function-based trajectory assignments for three time slices; these serve as the reference
\( D \)-values. Their sum, \( D_R = \sum_n D_n \) where \( n \) is each unique trajectory, is the initial total reference
cost for each frame triplet. If \( D_R \) is very close to zero, the original trajectory assignments are
likely to be correct and we proceed to the next frame triplet. Otherwise, alternate tracks with
potentially lower $D_R$ are constructed as follows. Tracks are examined pairwise, single particle swaps are instituted, and $D_R$ is recomputed. If the new value is larger than the reference, the swap is rejected; if it is smaller, the new observation IDs are accepted and the current $D_R$ becomes the reference $D_R$. This process is repeated for each time slice until a minimum $D_R$ is obtained or until all eligible swaps are exhausted. The computation is then repeated for the next frame.

**Validation Procedure for the Particle Tracker**

Performance of the algorithm was tested against simulated trajectories generated using the following inputs: the total number of objects generated over the course of a simulation $n_p$, the $x$- and $y$- dimensions of the “image,” a value which defines the typical time between successive objects entering the field of view or spawning within the field of view, which we refer to as the spawn rate, initial position $(x_i, y_i)$, the initial velocities $(v_{xi}, v_{yi})$, and the acceleration $(a_y)$ along the direction of the force of each object, and noise components drawn independently for $x$- and $y$-object coordinates from a Normal distribution with mean zero and variance $\sigma$. Object coordinates were determined using the basic kinematical equations:

$$x_{t2} = x_{t1} + (t_{2} - t_{1})v_{xt}$$

$$y_{t2} = y_{t1} + (t_{2} - t_{1})v_{yt} + \frac{a_y(t_{2} - t_{1})^2}{2}$$

Here, $x_{t1}$ is the position of the particle at time $t_1$, $x_{t2}$ its position at $t_2$, $v_{xt}$ its initial velocity along $x$, $v_{yt}$ its initial velocity along $y$, and $a_y$ its acceleration along $y$. (If an object spawns between two time steps, its motion to the next time step is determined using the kinematical equations.) Noise is then added, and the final output is a list of observations $(x, y, t)$ and trajectory ID numbers for
each observation.

The following spawn rates were tested: [1/16, 1/8, 1/4, 1/2, 1, 3/2, 2]. Spawn rates larger than 1 resulted in little change in performance so we tested only two larger values. The “image” dimensions were \( x = 400 \) and \( y = 800 \) for all simulations. The \( x_i \) values were drawn with uniform probability from the intervals: [200, 200], [150, 250], [100, 300], [50, 350], [1, 400]. The \( y_i \) were drawn from [1, 1], [1, 50], [1, 100], [1, 150], [1, 200]. The \( v_{xi} \) were set to \([0, 0], [-1, 1], [-2, 2], [-3, 3], [-4, 4] \); \( v_{yi} \) to \([0, 0]; [0, 2]; [0, 4]; [0, 6]; [0, 8]; a_y \) to \([9, 9], [7, 11], [5, 13], [3, 15], [1, 17] \). The Gaussian distributions were chosen with zero mean and variances \( \sigma_n = [0, 1, 2, 3] \) for both \( x \)- and \( y \)-coordinates. These input values are similar to experimental conditions, and the ranges were chosen to study the sensitivity of the algorithm to variations in the values of these parameters.

We also performed simulations in which the input parameters were chosen to recapitulate bead drop experiments. These typically capture around 100 particles so we set \( n_p = 100 \). Since the particles are injected from the micro-pipette in the manner of vertical line source we set \( x_i = [100, 100] \) and \( y_i = [1, 100] \), which resulted in particles spawning from a line one pixel wide and 100 pixels high. The magnetic beads have a horizontal velocity (\( x \)-direction) component; however, we found minimal initial velocity in the \( y \)-direction since the injection is perpendicular to the direction of the force: \( v_{xi} = [-0.5, 2.5] \) and \( v_{yi} = [0, 0] \). For the spawn rate, there are a few instances in which as many as 6 particles appear simultaneously out of the pipette, which would imply a rate of 1/6. However, most often particles may be separated by dozens of frames, which would be a rate of 12. Since we wanted to challenge the algorithm, we drew spawn rates from [1/6, 2]. Variations in the magnetic moments of the super-paramagnetic particles imply
variations in the magnetic forces on them. Thus, we expect a small fluctuation around a mean acceleration. Moreover, the mean acceleration depends on the distance from the magnet. Thus, simulations were run with $a_y = [1, 2]; [5, 6]; [9, 10]; [13, 14]; [17, 18]; [21, 22]; [25, 26]$. Simulations were also performed at the 4 different noise strengths described previously.

Comparison between the algorithm and simulations was performed by quantifying the number of correct individual links and by using the Variation of Information metric. For the former, the ID assignments in the algorithm output list are examined for each pair of consecutive time steps and each trajectory. These are matched to simulations. Pair-wise mismatches are scored as incorrect links; otherwise, the links are scored as correct. The Variation of Information metric is defined as follows for any two partitions $X$ and $Y$ of a set $A$; a partition consists of disjoint subsets $X = \{x_1, x_2, ... , x_e\}$ and $Y = \{y_1, y_2, ... , y_f\}$:

$$\text{VI}(X; Y) = - \sum_{i,j} r_{i,j} \left[ \log \left( \frac{r_{i,j}}{p_i} \right) + \log \left( \frac{r_{i,j}}{q_j} \right) \right]$$

where

$$p_i = \frac{|X_i|}{n}, q_j = \frac{|Y_j|}{n}, r_{i,j} = \frac{|X_i \cap Y_j|}{n}, \text{ and } n = \sum_i |X_i| + \sum_j |Y_j| = |A|.$$ 

If the two partitions are identical, $\text{VI}$ is zero; otherwise, $\text{VI}$ increases with increasing mismatches between the partitions. In our case, $A$ consists of all observations in the simulations; $X$ consists of trajectory assignments from the simulations, and $Y$ the algorithm’s trajectories; the disjoint subsets are the individual trajectories.

RESULTS

We investigated the performance of the algorithm by drawing simulation parameters at random from the intervals specified previously. In general, performance of the algorithm is excellent and
insensitive to variations in the parameters within a wide range: greater than 90% correct trajectory links identified when the spawn rate is < 4 objects/frame independent of other parameter settings. For higher spawn rates, the algorithm loses some performance; however, we find that only at very high spawn rates - greater than 10 spawns per frame, which would correspond to 300 spawns per second at a typical 30Hz frame rate - does performance suffer substantially, in some cases finding fewer than 60% trajectory links correctly. At these rates, performance also decreases with changes in the y-initial position, the x- and y-initial velocities, and noise strength. This is because higher values of these parameters increase the probability that trajectories may intertwine, and this effect is especially pronounced at high spawn rates. On the other hand, increasing values of the x-initial position lead to better trajectory determinations since we effectively decrease the density of trajectories. Thus, the results are insensitive to the total number of objects, and the spawn rate emerges as the parameter that affects algorithm performance in a significant way. Also, as expected, lower variances in parameter distributions generally lead to more similar trajectories for a group of objects, while increasing the variance leads to the opposite result.

For the simulations based on bead drop experiments, the results are plotted in Fig. S3. Again, the overall performance is excellent and insensitive to choices of the other parameters within a wide range. For instance, at an acceleration of 5.5 and zero noise, the percent correct trajectory links is about 92%, while at the highest acceleration of 25.5 that value is about 86%, a difference of 6%. The introduction of noise lowers performance somewhat (as expected) and decreases the sensitivity of results to changes in the acceleration: a mean acceleration of 5.5 at noise strength 3 has 74% correct trajectory links and higher accelerations are within 2% of that value. The outlier
occurs at mean object acceleration of 1.5 for which the percentage is substantially higher for zero noise and but decreases at high noise. When the performance is measured using the $VI$, the trends discussed above hold, but we find that the results are less sensitive to noise. For example, for a mean acceleration of 1.5, the $VI$ changes by 2 when noise strength is increased from zero to 3 while at mean acceleration of 25.5 the difference is less than 0.5.

We find that performance results are somewhat dependent on choice of metric. For instance, the difference in percent correct trajectory links between mean accelerations of 1.5 and 25.5 at noise strength of 3 is less than 10%, yet the difference in $VI$ for these parameters is much more significant ranging from $VI$ greater than 2 for mean acceleration of 1.5 to less than 1 for mean acceleration of 25.5. However, on the whole, performance trends are consistent when examined using either metric, and the noise strength has a much greater influence on algorithm performance at low accelerations than it does at high accelerations.

SUPPLEMENTARY REFERENCES


### TABLE S1: HORIZONTAL MAGNETIC TWEEZERS COMPONENTS

<table>
<thead>
<tr>
<th>Component</th>
<th>Vendor</th>
<th>Item</th>
<th>Price (USD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical Table with Active Pneumatic Isolation</td>
<td>Thorlabs</td>
<td>T46HK</td>
<td>$6700</td>
</tr>
<tr>
<td>Inverted Light Microscope</td>
<td>Nikon</td>
<td>Eclipse TS100</td>
<td>$8000</td>
</tr>
<tr>
<td>Objective, 40x 0.65NA</td>
<td>Olympus</td>
<td>RMS40X</td>
<td>$785</td>
</tr>
<tr>
<td>CCD Camera with 1394a Firewire Connection</td>
<td>Sony</td>
<td>XCD-U100</td>
<td>$2000</td>
</tr>
<tr>
<td>Electronic Linear Motorized Stage System</td>
<td>Zaber</td>
<td>3 × LSM Stage / 3 × XMCB Controller / 1 × XJOY 3 / Cables</td>
<td>$7262</td>
</tr>
<tr>
<td>Hydraulic Micromanipulator</td>
<td>Siskiyou</td>
<td>MX630L S3432</td>
<td>$4050</td>
</tr>
<tr>
<td>Structural Rail and Carriages</td>
<td>Thomson</td>
<td>521H15A300 (rail)</td>
<td>$480</td>
</tr>
<tr>
<td></td>
<td></td>
<td>511H15A0 (carriage)</td>
<td></td>
</tr>
<tr>
<td>Micromanipulator Platform</td>
<td>Sutter</td>
<td>MD54</td>
<td>$480</td>
</tr>
<tr>
<td>Syringe Pump (x2)</td>
<td>New Era Pump</td>
<td>NE-1000</td>
<td>$750 (x2)</td>
</tr>
<tr>
<td>Pipette Puller</td>
<td>Sutter</td>
<td>P-97</td>
<td>$8350</td>
</tr>
<tr>
<td>Vertical Puller</td>
<td>Narashige</td>
<td>PC-10</td>
<td>$2973</td>
</tr>
<tr>
<td>Pipette Microforge</td>
<td>WPI</td>
<td>MF-200</td>
<td>$3695</td>
</tr>
<tr>
<td>Glass Capillary, 1mm OD, 6in length</td>
<td>WPI</td>
<td>1B100-6</td>
<td>$65 (per 500)</td>
</tr>
<tr>
<td>Tygon Tubing</td>
<td>US Plastics</td>
<td>ND-100-80, 0.04ID</td>
<td>$212</td>
</tr>
<tr>
<td>Magnets (x10)</td>
<td>Indigo Instruments</td>
<td>N42, NeFeBr,</td>
<td>$0.14 (x10)</td>
</tr>
<tr>
<td>Item Description</td>
<td>Manufacturer/Model</td>
<td>Quantity</td>
<td>Price</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>--------------------------</td>
<td>----------</td>
<td>--------</td>
</tr>
<tr>
<td>Desktop PC System</td>
<td>HP Z230</td>
<td>1</td>
<td>$1200</td>
</tr>
<tr>
<td>External RAID storage</td>
<td>CFI B8283ERGG</td>
<td>1</td>
<td>$380</td>
</tr>
<tr>
<td>Hard Drives (x8)</td>
<td>Seagate ST2000DM001</td>
<td>8</td>
<td>$76 (x8)</td>
</tr>
<tr>
<td>MATLAB</td>
<td>Mathworks R2015a Academic</td>
<td>1</td>
<td>$500</td>
</tr>
<tr>
<td>LabView</td>
<td>National Instruments LabView 2014 SP1</td>
<td>1</td>
<td>$1000</td>
</tr>
<tr>
<td>Sample Cell Holder</td>
<td>Custom machined Aluminum stock</td>
<td>1</td>
<td>$50</td>
</tr>
<tr>
<td>Hydraulic Micromanipulator Mounting Bracket</td>
<td>Custom machined Aluminum stock</td>
<td>1</td>
<td>$40</td>
</tr>
<tr>
<td>Electronic Micromanipulator Mounting Bracket</td>
<td>Custom machined Aluminum stock</td>
<td>1</td>
<td>$25</td>
</tr>
<tr>
<td>Micropipette Holder</td>
<td>Custom machined Aluminum stock</td>
<td>1</td>
<td>$25</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>$50,381</strong></td>
</tr>
</tbody>
</table>

Where items are no longer produced or available, comparable items are specified. A partial list of consumables includes cover slips (#1), glass slides, RTV silicone sealant, syringes, and syringe needles.
Fig. S1. Pipette clamp.

The adapter used for clamping aspiration and microinjection micropipettes is shown. For physical dimensions, please see section 7 in Supplementary Materials.
Fig. S2. Photo of assembled tweezers.

Assembled tweezers components are shown. The motorized micromanipulator is in red border, the rail and carriage system in light green border, the sample cell in pink border and so forth. Not shown are the CCD sensor, illumination source, zoom lens, and syringe pumps. For dimensions, see the relevant sections in Supplementary Material; sizes can also be roughly gauged by comparison to the holes in the optical table (in silver) seen primarily at the extreme top and right sides of the image.
Fig. S3. Particle tracking performance for simulations of bead drop experiments.

These trials varied the mean acceleration as a way to symbolize the change in force on the super-paramagnetic micro-particles that were observed in the experiments. The mean acceleration values of 1.5, 5.5, 9.5, 13.5, 17.5, 21.5, and 25.5 were used - all in units of pixels/frame$^2$ - and are plotted on the $x$-axis. Due to the inhomogeneity of the super-paramagnetic micro-particles, around each mean acceleration value the variation spanned $\pm0.5$ pixel/frame$^2$. Thus, a mean acceleration of 1.5 could vary on the interval [1, 2] pixels/frame$^2$, a mean acceleration of 5.5 could vary on the interval [5, 6] pixels/frame$^2$, and so on. The other simulation input values for these trials were $n_p = 100$, $x_i = [100, 100]$, $y_i = [1, 100]$, $v_{xi} = [-0.5, 2.5]$, $v_{yi} = [0, 0]$, and $rate = [1/6, 2]$. All simulations were run with noise strengths of 0, 1, 2, and 3 as shown in the figure legend.