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H factor validation

The degree of volume overestimation for prolate spheroids can be estimated by using the known prolate spheroid drag/shape relationship,[1] which allows the spheroid Brownian motion to be calculated and compared to spheres of equivalent volume (Fig. S1). As the *r* increases, the Brownian motion overestimation is also seen to increase. This calculation assumes perfect imaging of the ellipsoids. In practice, the imaging is not perfect in large part due to camera pixelation.

Pixelation distortion tends to increase the observed image *H* as compared to the actual particle *H*. This is especially true with smaller particle images, which may only contain 10 pixels. To validate the use of *H*, we simulated the imaging of a prolate ellipsoid with defined *r* and maximal cross-sectional area (A_C) in an exhaustive variety of orientations and positions within the pixel grid. The probability of the ellipsoid being retained by the $H \leq 1.15$ cutoff during a 3 second observation time was then calculated (retention probability). This was repeated for a number of different *r* and A_C (over 10 million combinations were investigated). The results are summarized in Fig. S2. For large images, the *H* filter works as expected; ellipsoids with *r* greater than 1.85 are removed with high effieciency. For the smaller images, only the most spherical particles make it through the *H* filter with high efficiency. Apparently pixelation increases the selectivity of the *H* filter for the smaller images. Thus, using the $H \leq 1.15$ condition is even more accurate than naively expected (due to the enhanced filtering of the smaller particles).

To further validate the use of *H*, 1.898 µm beads were were flowed through the system and the mean *H* of individual tracks tabulated into a histogram (Fig. S3). This mean *H* histogram has a single peak at 1.015, which is slightly greater than 1 due to pixelation effects. As a more stringent test on the effects of pixelation, the maximum *H* of 456 nm beads tracks were also studied. This maximum *H* histogram was bimodal (Fig. S4), with the low *H* peak interpreted as coming from single beads and the high *H* peak assumed to come from multi-bead clusters. The low *H* peak lies within 1.00 to 1.15, further supporting the use of the $H \leq 1.15$ threshold.

1. J. S. McNown and J. Malaika, *Trans. Am. Geophys. Union*, 1950, **31**, 74-82.

Electronic Supplementary Material (ESI) for Analytical Methods
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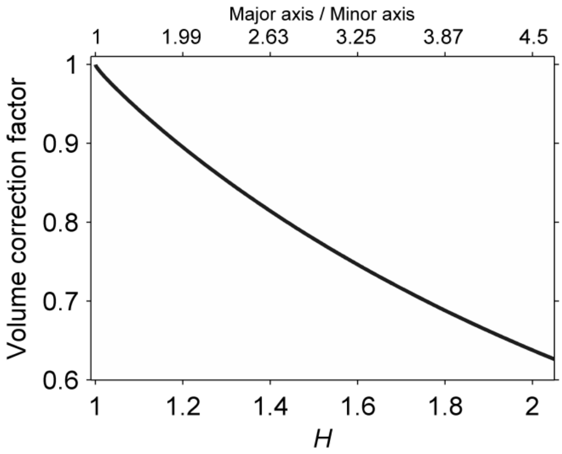

Fig. S1 Effect of prolate spheroid geometry on the Brownian motion volume estimator which assumes perfect spheres. For prolate spheroids, the volume is overestimated and must be multiplied by the volume correction factor. When the major axis/minor axis of the prolate spheroid is equal to 1, the prolate spheroid becomes a sphere and the volume correction factor is unity.

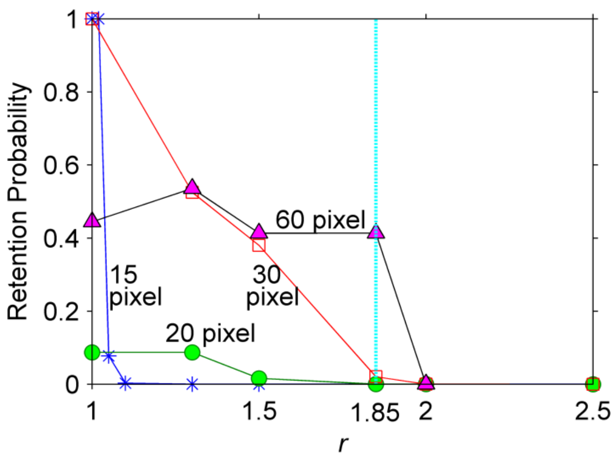

Fig. S2 Retention probability of prolate spheroids with an $H \leq 1.15$ filter condition (equivalent to $r \leq 1.85$; marked as a verticle blue line) imaged for 3 seconds as a function of ellipsoid r and A_C. The A_C for each set is labeled on the figure.

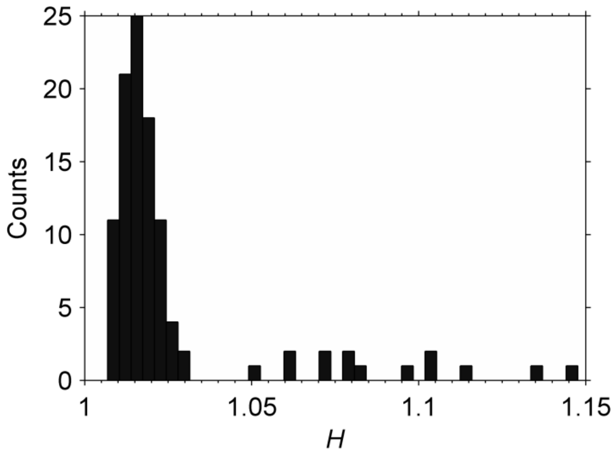

Fig. S3 Histogram of mean *H* for 1.898 µm polystyrene beads tracks in sizing buffer.

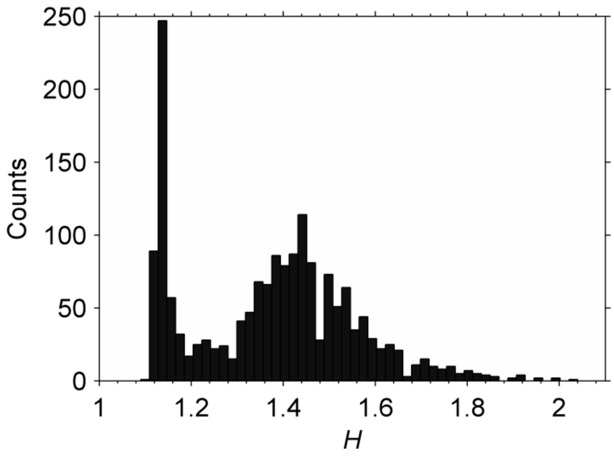

Fig. S4 Histogram of the maximum *H* for 456 nm polystyrene beads tracks in sizing buffer.