SUPPLEMENTAL MATERIAL

Chromosomal locus tracking with proper accounting of static and dynamic errors

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DERIVATION OF EQ. (4)

In this section we detail our derivation of the expression for the expectation value of the measured MSD, $E\left[\hat{M}(n)\right]$, of a particle undergoing FBM in the presence of photon noise and motion blurring during finite camera exposure time t_E. We assume that $x(\mathrm{t})$ is a fractional Brownian motion (in one dimension) for which $x(0)=0$. Thus we have the defining covariance relation:

$$E\left[x(t_1)x(t_2)\right]=D^*(t_1^\alpha+t_2^\alpha-|t_1-t_2|^\alpha) \tag{S1}$$

Our derivation follows the formalism used by Michalet in his treatment of pure Brownian motion [1], in which the estimated position during a given frame is the mean of the positions of the recorded photons in that frame. This formalism takes into account the effects of shot noise, but does not take into account additional noise due to background or pixelation. To ultimately include the effects of background and pixelation we amend our final result analogously to the approach applied for pure Brownian motion in [2]. The validity of this approach is confirmed by our simulations detailed in the main text.

Let $\hat{x}_k$ be the estimated position in the k$^{\text{th}}$ frame. Mathematically we have:

$$\hat{x}_k=\frac{1}{p}\sum_{i=1}^{p}\left[x_i^{(k)}+\xi_i^{(k)}\right] \tag{S2}$$

where $p \sim Poisson(\overline{p})$ is the number of photons recorded during the k$^{\text{th}}$ frame, $x_i^{(k)}$ is the position of the emitter at the time of the i$^{\text{th}}$ photon arrival of the k$^{\text{th}}$ frame, and $\xi_i^{(k)}$ is a RV that accounts for the noise in the detected photon position with $E\left[\xi_i^{(k)}\right]=0$ and $Var\left[\xi_i^{(k)}\right]=s_0^2$. Note that s_0 is the standard deviation of the microscope's Point Spread Function. Likewise we equate the position estimate of the (k+n)$^{\text{th}}$ frame:

$$\hat{x}_{k+n}=\frac{1}{q}\sum_{j=1}^{q}\left[x_j^{(k+n)}+\xi_j^{(k+n)}\right] \tag{S3}$$

where q and p are IID and $\xi_j^{(k+n)}$ and $\xi_i^{(k)}$ are IID Regardless of whether $M\hat{S}D(n)$ is computed via time-, ensemble-, or time-ensemble-averaging for this ergodic process, if we take track length and the number of tracks as fixed we have:

$$E\left[\hat{M}(n)\right] = \mathrm{E}\left[\left(\hat{x}_{k+n} - \hat{x}_k\right)^2\right] \tag{S4}$$

We substitute Eq. (S2) and (S3) into (S4) and expand:

$$\begin{aligned}
E\left[\hat{M}(n)\right] &= E\left[\left(\frac{1}{p}\sum_{i=1}^{p}\left(x_i^{(k)}+\xi_i^{(k)}\right) - \frac{1}{q}\sum_{j=1}^{q}\left(x_j^{(k+n)}+\xi_j^{(k+n)}\right)\right)^2\right] \\
&= E\left[\left(\frac{1}{p}\sum_{i=1}^{p}x_i^{(k)} - \frac{1}{q}\sum_{j=1}^{q}x_j^{(k+n)}\right)^2\right] + E\left[\frac{1}{p^2}\sum_{i=1}^{p}\left(\xi_i^{(k)}\right)^2\right] + E\left[\frac{1}{q^2}\sum_{j=1}^{q}\left(\xi_j^{(k+n)}\right)^2\right] \\
&= E\left[\left(\frac{1}{p}\sum_{i=1}^{p}x_i^{(k)} - \frac{1}{q}\sum_{j=1}^{q}x_j^{(k+n)}\right)^2\right] + E_p\left[\frac{1}{p^2}\sum_{i=1}^{p}{s_0}^2\right] + E_q\left[\frac{1}{q^2}\sum_{j=1}^{q}{s_0}^2\right] \\
&= E\left[\left(\frac{1}{p}\sum_{i=1}^{p}x_i^{(k)} - \frac{1}{q}\sum_{j=1}^{q}x_j^{(k+n)}\right)^2\right] + 2{s_0}^2 E_p\left(\frac{1}{p}\right)
\end{aligned} \tag{S5}$$

Strictly speaking, if p is truly Poisson then $E_p\left(\frac{1}{p}\right)$ will diverge since there is a finite probability that $p = 0$. However, in our context this would lead to a frame during which the particle is undetectable, a case which would be thrown out in the analysis. Thus we assume $p > 0$. This assumption changes the PMF of p slightly by a factor of $1/(1-e^{-\bar{p}})$, but we ignore the negligible effect this has on the positive moments of p. More importantly, this assumption allows us to enumerate $E_p\left(\frac{1}{p}\right)$ via an expression given in [3]:

$$\begin{aligned}
E_p\left(\frac{1}{p}\right) &= \sum_{r=1}^{\infty}\frac{(r-1)!}{\bar{p}^r} \\
&\approx \frac{1}{\bar{p}}
\end{aligned} \tag{S6}$$

where we have dropped terms $\mathcal{O}\left[\left(\frac{1}{\bar{p}}\right)^2\right]$. Letting $\sigma_0^2 = \frac{s_0^2}{\bar{p}}$, we then have the thus-far unsurprising result:

$$E\left[\hat{M}(n)\right] = E\left[\left(\frac{1}{p}\sum_{i=1}^{p} x_i^{(k)} - \frac{1}{q}\sum_{j=1}^{q} x_j^{(k_n)}\right)^2\right] + 2\sigma_0^2 \tag{S7}$$

Expanding the first term of Eq. (S7) now gives:

$$E\left[\hat{M}(n)\right] = 2\sigma_0^2 + E\left[\frac{1}{p^2}\sum_{i_1=1}^{p}\sum_{i_2=1}^{p} x_{i_1}^{(k)} x_{i_2}^{(k)}\right] + E\left[\frac{1}{q^2}\sum_{j_1=1}^{q}\sum_{j_2=1}^{q} x_{j_1}^{(k+n)} x_{j_2}^{(k+n)}\right] - 2E\left[\frac{1}{pq}\sum_{i=1}^{p}\sum_{j=1}^{q} x_i^{(k)} x_j^{(k+n)}\right] \tag{S8}$$

To proceed we employ the law of total expectation and take each expectation value in "layers", i.e. $E(\cdot) = \mathrm{E}_p\left(E_{t|p}\left(E_{x|t,p}(\cdot)\right)\right)$, whereby $E_p(\cdot)$ we mean the expectation w.r.t. all photon variables $\{p, q\}$, $E_{t|p}(\cdot)$ denotes the expectation w.r.t. all time variables $\{t_1^{(k)},\ldots,t_p^{(k)},t_1^{(k+n)},\ldots,t_q^{(k+n)}\}$ given the values of $\{p, q\}$, and $E_{x|t,p}(\cdot)$ denotes the expectation w.r.t. all position variables $\{x_1^{(k)},\ldots,x_p^{(k)},x_1^{(k+n)},\ldots,x_q^{(k+n)}\}$ given the values of $\{p, q\}$ and $\{t_1^{(k)},\ldots,t_p^{(k)},t_1^{(k+n)},\ldots,t_q^{(k+n)}\}$. Note that the time variable $t_i^{(k)}$ represents the time since the beginning of the k$^{\text{th}}$ frame (i.e. since $t = (k-1)t_E$) of the i$^{\text{th}}$ photon arrival during said frame. Thus we have:

$$\begin{aligned}
E\left[\hat{M}(n)\right] &= 2\sigma_0^2 + E\left[\frac{1}{p^2}\sum_{i_1=1}^{p}\sum_{i_2=1}^{p}E_{x|t,p}\left(x_{i_1}^{(k)}x_{i_2}^{(k)}\right)\right.\\
&+\frac{1}{q^2}\sum_{j_1=1}^{q}\sum_{j_2=1}^{q}E_{x|t,p}\left(x_{j_1}^{(k+n)}x_{j_2}^{(k+n)}\right)\\
&\left.-\frac{2}{pq}\sum_{i=1}^{p}\sum_{j=1}^{q}E_{x|t,p}\left(x_{i}^{(k)}x_{j}^{(k+n)}\right)\right]\\
&= 2\sigma_0^2 + D^{*}E\left[\frac{1}{p^2}\sum_{i_1=1}^{p}\sum_{i_2=1}^{p}\left[\left((k-1)t_E+t_{i_1}^{(k)}\right)^{\alpha}+\left((k-1)t_E+t_{i_2}^{(k)}\right)^{\alpha}-\left|t_{i_1}^{(k)}-t_{i_2}^{(k)}\right|^{\alpha}\right]\right.\\
&+\frac{1}{q^2}\sum_{j_1=1}^{q}\sum_{j_2=1}^{q}\left[\left((k+n-1)t_E+t_{j_1}^{(k+n)}\right)^{\alpha}+\left((k+n-1)t_E+t_{j_2}^{(k+n)}\right)^{\alpha}-\left|t_{j_1}^{(k+n)}-t_{j_2}^{(k+n)}\right|^{\alpha}\right]\\
&\left.\frac{1}{pq}\sum_{i=1}^{p}\sum_{j=1}^{q}\left[\left((k+n-1)t_E+t_{j}^{(k+n)}\right)^{\alpha}+\left((k-1)t_E+t_{i}^{(k)}\right)^{\alpha}-\left|nt_E+t_{j}^{(k+n)}-t_{i}^{(k)}\right|^{\alpha}\right]\right]
\end{aligned}\tag{S9}$$

where we have used the relation in Eq. (S1). The RHS of Eq. (S9) can be further simplified:

$$\begin{aligned}
E\left[\hat{M}(n)\right] &= 2\sigma_0^2 + D^{*}E\left[\frac{1}{p}\sum_{i_1=1}^{p}\left((k-1)t_E+t_{i_1}^{(k)}\right)^{\alpha}+\frac{1}{p}\sum_{i_2=1}^{p}\left((k-1)t_E+t_{i_2}^{(k)}\right)^{\alpha}-\frac{1}{p^2}\sum_{i_1=1}^{p}\sum_{i_2=1}^{p}\left|t_{i_1}^{(k)}-t_{i_2}^{(k)}\right|^{\alpha}\right.\\
&+\frac{1}{q}\sum_{j_1=1}^{q}\left((k+n-1)t_E+t_{j_1}^{(k+n)}\right)^{\alpha}+\frac{1}{q}\sum_{j_2=1}^{q}\left((k+n-1)t_E+t_{j_2}^{(k+n)}\right)^{\alpha}-\frac{1}{q^2}\sum_{j_1=1}^{q}\sum_{j_2=1}^{q}\left|t_{j_1}^{(k+n)}-t_{j_2}^{(k+n)}\right|^{\alpha}\\
&\left.-\frac{2}{p}\sum_{i=1}^{p}\left((k-1)t_E+t_{i}^{(k)}\right)^{\alpha}-\frac{2}{q}\sum_{j=1}^{q}\left((k+n-1)t_E+t_{j}^{(k+n)}\right)^{\alpha}+\frac{2}{pq}\sum_{i=1}^{p}\sum_{j=1}^{q}\left|nt_E+t_{j}^{(k+n)}-t_{i}^{(k)}\right|^{\alpha}\right]\\
&= 2\sigma_0^2 + D^{*}\left\{2E\left[\frac{1}{p}\sum_{i=1}^{p}\left((k-1)t_E+t_{i}^{(k)}\right)^{\alpha}\right]-E\left[\frac{1}{p^2}\sum_{i_1=1}^{p}\sum_{i_2=1}^{p}\left|t_{i_1}^{(k)}-t_{i_2}^{(k)}\right|^{\alpha}\right]\right.\\
&+2E\left[\frac{1}{q}\sum_{j=1}^{q}\left((k+n-1)t_E+t_{j}^{(k+n)}\right)^{\alpha}\right]-E\left[\frac{1}{q^2}\sum_{j_1=1}^{q}\sum_{j_2=1}^{q}\left|t_{j_1}^{(k+n)}-t_{j_2}^{(k+n)}\right|^{\alpha}\right]\\
&-2E\left[\frac{1}{p}\sum_{i=1}^{p}\left((k-1)t_E+t_{i}^{(k)}\right)^{\alpha}\right]-2E\left[\frac{1}{q}\sum_{j=1}^{q}\left((k+n-1)t_E+t_{j}^{(k+n)}\right)^{\alpha}\right]\\
&\left.+2E\left[\frac{1}{pq}\sum_{i=1}^{p}\sum_{j=1}^{q}\left|nt_E+t_{j}^{(k+n)}-t_{i}^{(k)}\right|^{\alpha}\right]\right\}
\end{aligned}\tag{S10}$$

Various terms in Eq. (S10) cancel to give:

$$
\begin{aligned}
E\left[\hat{M}(n)\right] &= 2\sigma_0^2 + 2D^* E\left[\frac{1}{pq}\sum_{i=1}^{p}\sum_{j=1}^{q}\left|nt_E + t_j^{(k+n)} - t_i^{(k)}\right|^\alpha\right] \\
&\quad - D^* E\left[\frac{1}{p^2}\sum_{i_1=1}^{p}\sum_{i_2=1}^{p}\left|t_{i_1}^{(k)} - t_{i_2}^{(k)}\right|^\alpha\right] \\
&\quad - D^* E\left[\frac{1}{q^2}\sum_{j_1=1}^{q}\sum_{j_2=1}^{q}\left|t_{j_1}^{(k+n)} - t_{j_2}^{(k+n)}\right|^\alpha\right]
\end{aligned} \tag{S11}
$$

Furthermore, the last two terms of Eq. (S11) are equivalent since $\left(t_i^{(k)} \mid p\right) \sim \left(t_i^{(k+n)} \mid q\right)$ and $p \sim q$. Thus:

$$
\begin{aligned}
E\left[\hat{M}(n)\right] &= 2\sigma_0^2 + 2D^* E\left[\frac{1}{pq}\sum_{i=1}^{p}\sum_{j=1}^{q}\left|nt_E + t_j^{(k+n)} - t_i^{(k)}\right|^\alpha\right] \\
&\quad - 2D^* E\left[\frac{1}{p^2}\sum_{i_1=1}^{p}\sum_{i_2=1}^{p}\left|t_{i_1}^{(k)} - t_{i_2}^{(k)}\right|^\alpha\right]
\end{aligned} \tag{S12}
$$

We must now find the two expectation terms in Eq. (S12). We start with the second one, giving:

$$
\begin{aligned}
E\left[\frac{1}{p^2}\sum_{i_1=1}^{p}\sum_{i_2=1}^{p}\left|t_{i_1}^{(k)} - t_{i_2}^{(k)}\right|^\alpha\right] &= E_p\left[\frac{1}{p^2}\sum_{i_1=1}^{p}\sum_{i_2=1}^{p}E_{t|p}\left(\left|t_{i_1}^{(k)} - t_{i_2}^{(k)}\right|^\alpha\right)\right] \\
&= 2E_p\left[\frac{1}{p^2}\sum_{i_1=2}^{p}\sum_{i_2=1}^{i_1-1}E_{t|p}\left(\left[t_{i_1}^{(k)} - t_{i_2}^{(k)}\right]^\alpha\right)\right]
\end{aligned} \tag{S13}
$$

where we have re-indexed to ensure $i_1 > i_2$. Since $\left\{t_1^{(k)}, t_2^{(k)}, \ldots\right\}$ are ordered statistics this also implies $t_{i_1}^{(k)} > t_{i_2}^{(k)}$ and thus we drop the absolute value sign in the second line. For brevity of notation we will drop the superscript "(k)" for the rest of this part of the derivation. The innermost expectation value of Eq. (S13) can be written explicitly in terms of the conditional probability distribution function $f_{t_{i_1}, t_{i_2}|p}\left(t_{i_1}, t_{i_2} \mid p\right)$:

$$
E_{t|p}\left[\left(t_{i_1} - t_{i_2}\right)^\alpha\right] = \int_0^{t_E}\int_0^{t_{i_1}}\left(t_{i_1} - t_{i_2}\right)^\alpha f_{t_{i_1}, t_{i_2}|p}\left(t_{i_1}, t_{i_2} \mid p\right) dt_{i_2} dt_{i_1} \tag{S14}
$$

Thus we need to specify this PDF. Since $t_{i_1}, t_{i_2} \mid p$ are order statistics of uniform random variables on the interval $(0, t_E)$ their joint distribution has a known form [4]:

$$f_{t_{i_1},t_{i_2}|p}\left(t_{i_1},t_{i_2}\mid p\right)=\frac{p!}{t_E^p(i_2-1)!(i_1-i_2-1)!(p-i_1)!}t_{i_2}^{i_2-1}\left(t_{i_1}-t_{i_2}\right)^{i_1-i_2-1}\left(t_E-t_{i_1}\right)^{p-i_1} \quad \text{(S15)}$$

To further simplify notation, let $u=t_{i_1}, v=t_{i_2}, a=i_1, b=i_2$:

$$\begin{aligned}E_{t|p}\left[\left(t_{i_1}-t_{i_2}\right)^{\alpha}\right] &=\frac{p!}{t_E^p(b-1)!(a-b-1)!(p-a)!}\int_0^{t_E}\int_0^u (u-v)^{\alpha}v^{b-1}(u-v)^{a-b-1}(t_E-u)^{p-a}\,dvdu\\ &\frac{p!t_E^{\alpha-2}}{(b-1)!(a-b-1)!(p-a)!}\int_0^{t_E}\int_0^u\left(\frac{u-v}{t_E}\right)^{\alpha}\left(\frac{v}{t_E}\right)^{b-1}\left(\frac{u-v}{t_E}\right)^{a-b-1}\left(1-\frac{u}{t_E}\right)^{p-a}dvdu\end{aligned}$$

(S16)

Making the substitutions $\frac{u}{t_E}\mapsto u, \frac{v}{t_E}\mapsto v$:

$$E_{t|p}\left[\left(t_{i_1}-t_{i_2}\right)^{\alpha}\right]=\frac{p!t_E^{\alpha}}{(b-1)!(a-b-1)!(p-a)!}\int_0^1\int_0^u (u-v)^{\alpha+a-b-a}v^{b-1}(1-u)^{p-a}\,dvdu \quad \text{(S17)}$$

First we compute the innermost integral in Eq. (S17):

$$\int_0^u (u-v)^{\alpha+a-b-1}v^{b-1}dv=u^{\alpha+a-2}\int_0^u\left(1-\frac{v}{u}\right)^{\alpha+a-b-1}\left(\frac{v}{u}\right)^{b-1}dv \quad \text{(S18)}$$

Letting $x=\frac{v}{u}$:

$$\int_0^u (u-v)^{\alpha+a-b-1}v^{b-1}dv=u^{\alpha+a-1}\int_0^1(1-x)^{\alpha+a-b-1}x^{b-1}dx \quad \text{(S19)}$$

Note that the integral on the RHS of Eq. (S19) is a Beta function:

$$\int_0^u (u-v)^{\alpha+a-b-1}v^{b-1}dv=u^{\alpha+a-1}B(\alpha+a-b,b) \quad \text{(S20)}$$

Plugging Eq. (S20) into Eq. (S17) gives:

$$\begin{aligned}E_{t|p}\left[\left(t_{i_1}-t_{i_2}\right)^{\alpha}\right] &=\frac{t_E^{\alpha}p!B(\alpha+a-b,b)}{(b-1)!(a-b-1)!(p-a)!}\int_0^1 u^{\alpha+a-1}(1-u)^{p-a}du\\ &=t_E^{\alpha}\frac{p!}{(b-1)!(a-b-1)!(p-a)!}B(\alpha+a-b,b)B(\alpha+a,p-a+1)\end{aligned} \quad \text{(S21)}$$

Now let’s plug Eq. (S21) back into the double sum of Eq. (S13):

$$\sum_{a=2}^{p}\sum_{b=1}^{a-1} E_{t|p}\left[(u-v)^{\alpha}\right] = t_E^{\alpha}\sum_{a=2}^{p}\frac{p!B(\alpha+a,p-a+1)}{(p-a)!}\sum_{b=1}^{a-1}\frac{1}{(b-1)!(a-b-1)!}B(\alpha+a-b,b)$$
$$= t_E^{\alpha}\sum_{a=2}^{p}\frac{p!B(\alpha+a,p-a+1)}{(a-2)!(p-a)!}\sum_{b=1}^{a-1}\frac{(a-2)!}{(b-1)!(a-b-1)!}B(\alpha+a-b,b) \quad \text{(S22)}$$

Consider the rightmost sum in Eq. (S22):

$$\sum_{b=1}^{a-1}\frac{(a-2)!}{(b-1)!(a-b-1)!}B(\alpha+a-b,b)=\sum_{b=1}^{a-1}\binom{a-2}{b-1}B(\alpha+a-b,b) \quad \text{(S23)}$$

We re-index and let $k=b-1$ and $n=a-2$ to put Eq. (S23) into a suggestive form for further simplification:

$$\sum_{b=1}^{a-1}\binom{a-2}{b-1}B(\alpha+a-b,b) = \sum_{k=0}^{n}\binom{n}{k}B(\alpha+n-k+1,k+1)$$
$$= B(\alpha+1,1)\sum_{k=0}^{n}\binom{n}{k}\frac{B(\alpha+1+n-k,k+1)}{B(\alpha+1,1)} \quad \text{(S24)}$$
$$= B(\alpha+1,1)$$

where we have noted that $\binom{n}{k}\frac{B(\alpha+1+n-k,k+1)}{B(\alpha+1,1)}$ represents a normalized Beta-binomial distribution [5] and the sum in Eq. (S24) is over its full support. Note that $B(\alpha+1,1)=\frac{1}{\alpha+1}$.

Now Eq. (S22) becomes:

$$\sum_{a=2}^{p}\sum_{b=1}^{a-1} E_{t|p}\left[(u-v)^{\alpha}\right] = \frac{t_E^{\alpha}}{\alpha+1}\sum_{a=2}^{p}\frac{p!}{(a-2)!(p-a)!}B(\alpha+a,p-a+1)$$
$$= \frac{t_E^{\alpha}p(p-1)}{\alpha+1}\sum_{a=2}^{p}\frac{(p-2)!}{(a-2)!(p-a)!}B(\alpha+a,p-a+1) \quad \text{(S25)}$$
$$= \frac{t_E^{\alpha}p(p-1)}{\alpha+1}\sum_{a=2}^{p}\binom{p-2}{a-2}B(\alpha+a,p-a+1)$$

Now let $k=a-2$ and $n=p-2$ and re-index to again put the expressions into a suggestive form:

$$\begin{aligned}
\sum_{a=2}^{p}\sum_{b=1}^{a-1} E_{t|p}\left[(u-v)^{\alpha}\right] &= \frac{t_E^{\alpha}\, p(p-1)}{\alpha+1}\sum_{k=0}^{n}\binom{n}{k} B(\alpha+2+k, n-k+1) \\
&= \frac{t_E^{\alpha}\, p(p-1)}{\alpha+1} B(\alpha+2,1)\sum_{k=0}^{n}\binom{n}{k}\frac{B(\alpha+2+k, n-k+1)}{B(\alpha+2,1)} \\
&= \frac{t_E^{\alpha}\, p(p-1)}{\alpha+1} B(\alpha+2,1) \\
&= \frac{t_E^{\alpha}\, p(p-1)}{(\alpha+1)(\alpha+2)}
\end{aligned} \tag{S26}$$

where again we have recognized the normalized Beta-binomial distribution. Referring back to Eq. (S13) we now have:

$$\begin{aligned}
E\left[\frac{1}{p^2}\sum_{i_1=1}^{p}\sum_{i_2=1}^{p}\left|t_{i_1}^{(k)}-t_{i_2}^{(k)}\right|^{\alpha}\right] &= 2E_p\left[\frac{1}{p^2}\frac{t_E^{\alpha}\, p(p-1)}{(\alpha+2)(\alpha+1)}\right] \\
&= \frac{2t_E^{\alpha}}{(\alpha+2)(\alpha+1)} E_p\left(\frac{p-1}{p}\right) \\
&= \frac{2t_E^{\alpha}}{(\alpha+2)(\alpha+1)}\left(1-E_p\left(\frac{1}{p}\right)\right) \\
&\approx \frac{2t_E^{\alpha}}{(\alpha+2)(\alpha+1)}\left(1-\frac{1}{\overline{p}}\right)
\end{aligned} \tag{S27}$$

where in the last step we have again used the approximation due to [3]. Now we can update Equation S12 to give:

$$E\left[\hat{M}(n)\right] = 2\sigma_0^2 - \frac{4D^{*}t_E^{\alpha}}{(\alpha+1)(\alpha+2)}\left(1-\frac{1}{\overline{p}}\right) + 2D^{*}E\left[\frac{1}{pq}\sum_{i=1}^{p}\sum_{j=1}^{q}\left|nt_E + t_j^{(k+n)} - t_i^{(k)}\right|^{\alpha}\right] \tag{S28}$$

So we must now find the remaining expectation value. Note that the quantity within the absolute value in Eq. (S28) is nonnegative since $\left|t_j^{(k+n)} - t_i^{(k)}\right| \le t_E$ and $n \ge 1$. Thus:

$$E\left[\frac{1}{pq}\sum_{i=1}^{p}\sum_{j=1}^{q}\left|nt_E + t_j^{(k+n)} - t_i^{(k)}\right|^{\alpha}\right] = E_{p,q}\left[\frac{1}{pq}\sum_{i=1}^{p}\sum_{j=1}^{q}E_{t|p,q}\left(\left[nt_E + t_j^{(k+n)} - t_i^{(k)}\right]^{\alpha}\right)\right] \tag{S29}$$

We can write the innermost conditional expectation on the RHS of Eq. (S29) explicitly in terms of the relevant joint conditional PDF:

$$E_{t|p,q}\left[\left(nt_E + t_j^{(k+n)} - t_i^{(k+n)}\right)^\alpha\right] = \int_0^{t_E}\int_0^{t_E}(nt_E + u - v)^\alpha f_{t_j^{(k+n)},t_i^{(k)}|p,q}(u,v\,|\,p,q)\,dudv \qquad \text{(S30)}$$

Note that $\left(t_j^{(k+n)}\,|\,q\right)$ and $\left(t_i^{(k)}\,|\,p\right)$ are independent and so

$$\begin{aligned} f_{t_j^{(k+n)},t_i^{(k)}|p,q}(u,v\,|\,p,q) &= f_{t_j^{(k+n)}|q}(u)\, f_{t_i^{(k)}|p}(v) \\ &= \left[\frac{q}{t_E^q}\binom{q-1}{j-1}u^{j-1}(t_E-u)^{q-j}\right]\left[\frac{p}{t_E^p}\binom{p-1}{i-1}v^{i-1}(t_E-v)^{p-i}\right] \end{aligned} \qquad \text{(S31)}$$

where we have substituted the known PDF of an order statistic of a uniform random variable on $(0,t_E)$ [4]. Referring to Eq. (S30) we now must compute the integral:

$$\begin{aligned} E_{t|p,q}\left[\left(nt_E + t_j^{(k+n)} - t_i^{(k+n)}\right)^\alpha\right] &= \frac{pq}{t_E^{p+q}}\binom{p-1}{i-1}\binom{q-1}{j-1}\int_0^{t_E}\int_0^{t_E}(nt_E+u-v)^\alpha u^{j-1}(t_E-u)^{q-j}v^{i-1}(t_E-v)^{p-i}\,dudv \\ &= t_E^{\alpha+2}pq\binom{p-1}{i-1}\binom{q-1}{j-1}\times \\ &\qquad \int_0^{t_E}\int_0^{t_E}\left(n+\frac{u-v}{t_E}\right)^\alpha\left(\frac{u}{t_E}\right)^{j-1}\left(1-\frac{u}{t_E}\right)^{q-j}\left(\frac{v}{t_E}\right)^{i-1}\left(1-\frac{v}{t_E}\right)^{p-i}dudv \\ &= t_E^\alpha pq\binom{p-1}{i-1}\binom{q-1}{j-1}\int_0^1\int_0^1(n+u-v)^\alpha u^{j-1}(1-u)^{q-j}v^{i-1}(1-v)^{p-i}\,dudv \end{aligned}$$

(S32)

To proceed we expand $(n+u-v)^\alpha$ using the binomial theorem:

$$\begin{aligned} (n+u-v)^\alpha &= \sum_{k=0}^{\infty}\binom{\alpha}{k}n^{\alpha-k}(u-v)^k \\ &= \sum_{k=0}^{\infty}\binom{\alpha}{k}n^{\alpha-k}\sum_{m=0}^{k}\binom{k}{m}u^{k-m}v^m(-1)^m \end{aligned} \qquad \text{(S33)}$$

Plugging Eq. (S33) into Eq. (S32) and swapping the integrals and sums gives:

$$
\begin{aligned}
E_{t|p,q}\left[\left(nt_E+t_j^{(k+n)}-t_i^{(k+n)}\right)^\alpha\right] &= t_E^\alpha pq\binom{p-1}{i-1}\binom{q-1}{j-1}\sum_{k=0}^{\infty}\binom{\alpha}{k}n^{\alpha-k}\sum_{m=0}^{k}\binom{k}{m}(-1)^m \\
&\quad\times\left(\int_0^1 u^{k-m+j-1}(1-u)^{q-j}\,du\right) \\
&\quad\times\left(\int_0^1 v^{m+i-1}(1-v)^{p-i}\,dv\right) \\
&= t_E^\alpha pq\binom{p-1}{i-1}\binom{q-1}{j-1}\sum_{k=0}^{\infty}\binom{\alpha}{k}n^{\alpha-k}\sum_{m=0}^{k}\binom{k}{m}(-1)^m B(k-m+j,q-j+1) \\
&\quad\times B(m+i,p-i+1)
\end{aligned}
\tag{S34}
$$

We now plug Eq. (S34) into Eq. (S29), rearrange the sums, and begin to simplify.

$$
\begin{aligned}
E\left[\frac{1}{pq}\sum_{i=1}^{p}\sum_{j=1}^{q}\left|nt_E+t_j^{(k+n)}-t_i^{(k)}\right|^\alpha\right] &= E_{p,q}\left[\sum_{i=1}^{p}\sum_{j=1}^{q}t_E^\alpha\binom{p-1}{i-1}\binom{q-1}{j-1}\sum_{k=0}^{\infty}\binom{\alpha}{k}n^{\alpha-k}\right. \\
&\quad\left.\times\sum_{m=0}^{k}\binom{k}{m}(-1)^m B(k-m+j,q-j+1)B(m+i,p-i+1)\right] \\
&= t_E^\alpha\sum_{k=0}^{\infty}\binom{\alpha}{k}n^{\alpha-k}\sum_{m=0}^{k}\binom{k}{m}(-1)^m \\
&\quad\times E_{p,q}\left[\sum_{i=1}^{p}\sum_{j=1}^{q}\binom{p-1}{i-1}\binom{q-1}{j-1}B(k-m+j,q-j+1)B(m+i,p-i+1)\right]
\end{aligned}
\tag{S35}
$$

Consider the double sum in the square brackets of the RHS of the last line in Eq. (S35).

$$
\begin{aligned}
&\sum_{i=1}^{p}\sum_{j=1}^{q}\binom{p-1}{i-1}\binom{q-1}{j-1}B(k-m+j,q-j+1)B(m+i,p-i+1) \\
&= \left(\sum_{i=1}^{p}\binom{p-1}{i-1}B(m+i,p-i+1)\right)\left(\sum_{j=1}^{q}\binom{q-1}{j-1}B(k-m+j,q-j+1)\right) \\
&= \left(\sum_{i'=0}^{p'}\binom{p'}{i'}B(m+1+i',p'-i'+1)\right)\left(\sum_{j'=0}^{q'}\binom{q'}{j'}B(k-m+1+j',q'-j'+1)\right) \\
&= B(m+1,1)B(k-m+1,1) \\
&= \frac{1}{(m+1)(k-m+1)}
\end{aligned}
\tag{S36}
$$

where again we have recognized a sum over the Beta-binomial PMF. Plugging Eq. (S36) into Eq. (S35) now gives:

$$E\left[\frac{1}{pq}\sum_{i=1}^{p}\sum_{j=1}^{q}\left|nt_E + t_j^{(k+n)} - t_i^{(k)}\right|^{\alpha}\right] = t_E^{\alpha}\sum_{k=0}^{\infty}\binom{\alpha}{k} n^{\alpha-k}\sum_{m=0}^{k}\binom{k}{m}(-1)^m \frac{1}{(m+1)(k-m+1)} \tag{S37}$$

The sum over *m* in Eq. (S37) has a closed-form solution. Plugging it into Mathematica gives:

$$\sum_{m=0}^{k}\binom{k}{m}(-1)^m \frac{1}{(m+1)(k-m+1)} = \frac{1+(-1)^k}{(k+2)(k+1)} \tag{S38}$$

Hence,

$$E\left[\frac{1}{pq}\sum_{i=1}^{p}\sum_{j=1}^{q}\left|nt_E + t_j^{(k+n)} - t_i^{(k)}\right|^{\alpha}\right] = t_E^{\alpha}\sum_{k=0}^{\infty}\binom{\alpha}{k} n^{\alpha-k}\left(\frac{1+(-1)^k}{(k+2)(k+1)}\right) \tag{S39}$$

We can simplify Eq. (S39) further:

$$t_E^{\alpha}\sum_{k=0}^{\infty}\binom{\alpha}{k} n^{\alpha-k}\left(\frac{1+(-1)^k}{(k+2)(k+1)}\right) = \frac{t_E^{\alpha}}{(\alpha+2)(\alpha+1)}\left[\sum_{k=0}^{\infty}\binom{\alpha+2}{k+2} n^{\alpha-k} + \sum_{k=0}^{\infty}\binom{\alpha+2}{k+2} n^{\alpha-k}(-1)^k\right] \tag{S40}$$

Re-index Eq. (S40) with $k' = k+2$:

$$\begin{aligned}
t_E^{\alpha}\sum_{k=0}^{\infty}\binom{\alpha}{k} n^{\alpha-k}\left(\frac{1+(-1)^k}{(k+2)(k+1)}\right) &= \frac{t_E^{\alpha}}{(\alpha+2)(\alpha+1)}\left[\sum_{k'=2}^{\infty}\binom{\alpha+2}{k'} n^{\alpha+2-k'} + \sum_{k'=2}^{\infty}\binom{\alpha+2}{k'} n^{\alpha+2-k'}(-1)^{k'}\right] \\
&= \frac{t_E^{\alpha}}{(\alpha+2)(\alpha+1)}\left[\sum_{k'=0}^{\infty}\binom{\alpha+2}{k'} n^{\alpha+2-k'} - n^{\alpha+2} - (\alpha+2)n^{\alpha+1}\right. \\
&\qquad \left. + \sum_{k'=0}^{\infty}\binom{\alpha+2}{k'} n^{\alpha+2-k'}(-1)^{k'} - n^{\alpha+2} + (\alpha+2)n^{\alpha+1}\right] \\
&= \frac{t_E^{\alpha}}{(\alpha+2)(\alpha+1)}\left[\sum_{k'=0}^{\infty}\binom{\alpha+2}{k'} n^{\alpha+2-k'} + \sum_{k'=0}^{\infty}\binom{\alpha+2}{k'} n^{\alpha+2-k'}(-1)^{k'} - 2n^{\alpha+2}\right]
\end{aligned} \tag{S41}$$

Finally, we use the binomial theorem once again:

$$t_E^{\alpha}\sum_{k=0}^{\infty}\binom{\alpha}{k}n^{\alpha-k}\left(\frac{1+(-1)^k}{(k+2)(k+1)}\right)=\frac{t_E^{\alpha}}{(\alpha+2)(\alpha+1)}\left[(n+1)^{\alpha+2}+(n-1)^{\alpha+2}-2n^{\alpha+2}\right] \tag{S42}$$

And now we refer back to Eq. (S28) to give our ultimate result:

$$\begin{aligned} E\left[\hat{M}(n)\right] &= \frac{2D^{*}t_E^{\alpha}}{(\alpha+2)(\alpha+1)}\left[(n+1)^{\alpha+2}+(n-1)^{\alpha+2}-2n^{\alpha+2}\right] \\ &\quad +2\sigma_0^2+\frac{4D^{*}t_E^{\alpha}}{(\alpha+2)(\alpha+1)\,\overline{p}}-\frac{4D^{*}t_E^{\alpha}}{(\alpha+2)(\alpha+1)} \end{aligned} \tag{S43}$$

DERIVATION OF EQS. (20-22)

We now give our derivation of the expected value of the VAC of an FBM. This derivation makes the same assumptions as in our derivation of the MSD. The expected value of the VAC is defined by

$$E\left[\hat{C}_v^{(n)}(m)\right]=\frac{E\left[\left(\hat{x}_{k+n}-\hat{x}_k\right)\left(\hat{x}_{k+n+m}-\hat{x}_{k+m}\right)\right]}{(nt_E)^2} \tag{S44}$$

There are cases to be considered: $m = 0$, $m = n$, and $m \neq 0, n$. When m = 0 the VAC is proportional to the MSD via Eq. (15) and so we have:

$$\begin{aligned} E\left[\hat{C}_v^{(n)}(m=0)\right] &= \frac{2D^{*}t_E^{\alpha}}{(\alpha+2)(\alpha+1)(\mathrm{n}t_E)^2}\left[(n+1)^{\alpha+2}+(n-1)^{\alpha+2}-2n^{\alpha+2}\right] \\ &\quad +\frac{2\sigma_0^2}{(nt_E)^2}+\frac{4D^{*}t_E^{\alpha}}{(\alpha+2)(\alpha+1)(\mathrm{n}t_E)^2\,\overline{p}}-\frac{4D^{*}t_E^{\alpha}}{(\alpha+2)(\alpha+1)(\mathrm{n}t_E)^2} \end{aligned} \tag{S45}$$

Using the definition of σ from Eq. (6) and $A(u)$ from Eq. (19), we can rewrite in a more compact form:

$$E\left[\hat{C}_v^{(n)}(m=0)\right]=\frac{1}{(nt_E)^2}\left[\frac{2D^{*}t_E^{\alpha}\left(A(n)-2\right)}{(\alpha+2)(\alpha+1)}+2\sigma^2\right] \tag{S46}$$

which is the same as Eq. (20). Continuing with the more general situation, we need

$$
\begin{aligned}
E\left[\hat{C}_v^{(n)}(m)\right](nt_E)^2 &= E\left[\left(\frac{1}{q}\sum_{j=1}^{q}\left(x_j^{(k+n)}+\xi_j^{(k+n)}\right)-\frac{1}{p}\sum_{i=1}^{p}\left(x_i^{(k)}+\xi_i^{(k)}\right)\right)\times\right.\\
&\left.\left(\frac{1}{q'}\sum_{j'=1}^{q'}\left(x_{j'}^{(k+m+n)}+\xi_{j'}^{(k+m+n)}\right)-\frac{1}{p'}\sum_{i'=1}^{p'}\left(x_{i'}^{(k+m)}+\xi_{i'}^{(k+m)}\right)\right)\right]\\
&= E\left[\left(\frac{1}{q}\sum_{j=1}^{q}x_j^{(k+n)}-\frac{1}{p}\sum_{i=1}^{p}x_i^{(k)}\right)\left(\frac{1}{q'}\sum_{j'=1}^{q'}x_{j'}^{(k+m+n)}-\frac{1}{p'}\sum_{i'=1}^{p'}x_{i'}^{(k+m)}\right)\right]\\
&\quad +E\left[\frac{1}{pp'}\sum_{i'=1}^{p'}\sum_{i=1}^{p}\xi_i^{(k)}\xi_{i'}^{(k+m)}+\frac{1}{qq'}\sum_{j'=1}^{q'}\sum_{j=1}^{q}\xi_j^{(k+n)}\xi_{j'}^{(k+m+n)}\right.\\
&\quad\left.-\frac{1}{qp'}\sum_{i'=1}^{p'}\sum_{j=1}^{q}\xi_j^{(k+m)}\xi_{i'}^{(k+m)}-\frac{1}{pq'}\sum_{j'=1}^{q'}\sum_{i=1}^{p}\xi_i^{(k)}\xi_{j'}^{(k+m+n)}\right]\\
&= E\left[\left(\frac{1}{q}\sum_{j=1}^{q}x_j^{(k+n)}-\frac{1}{p}\sum_{i=1}^{p}x_i^{(k)}\right)\left(\frac{1}{q'}\sum_{j'=1}^{q'}x_{j'}^{(k+m+n)}-\frac{1}{p'}\sum_{i'=1}^{p'}x_{i'}^{(k+m)}\right)\right]\\
&\quad +s_0^2E\left[\frac{\delta_{k+m,k}}{p}+\frac{\delta_{k+m+n,k+n}}{q}-\frac{\delta_{k+m,k+n}}{q}-\frac{\delta_{k,k+m+n}}{p}\right]\\
&= E\left[\left(\frac{1}{q}\sum_{j=1}^{q}x_j^{(k+n)}-\frac{1}{p}\sum_{i=1}^{p}x_i^{(k)}\right)\left(\frac{1}{q'}\sum_{j'=1}^{q'}x_{j'}^{(k+m+n)}-\frac{1}{p'}\sum_{i'=1}^{p'}x_{i'}^{(k+m)}\right)\right]\\
&\quad +\sigma_0^2\left(\delta_{k+m,k}+\delta_{k+m+n,k+n}-\delta_{k+m,k+n}-\delta_{k,k+m+n}\right)\\
&= E\left[\left(\frac{1}{q}\sum_{j=1}^{q}x_j^{(k+n)}-\frac{1}{p}\sum_{i=1}^{p}x_i^{(k)}\right)\left(\frac{1}{q'}\sum_{j'=1}^{q'}x_{j'}^{(k+m+n)}-\frac{1}{p'}\sum_{i'=1}^{p'}x_{i'}^{(k+m)}\right)\right]+\begin{cases}2\sigma_0^2 & ,m=0\\ -\sigma_0^2 & ,m=n\\ 0 & else\end{cases}
\end{aligned}
\tag{S47}
$$

Now we must unwrap the remaining expectation value in the RHS of Eq. (S47). Expanding, we have:

$$
\begin{aligned}
&E\left[\left(\frac{1}{q}\sum_{j=1}^{q}x_j^{(k+n)}-\frac{1}{p}\sum_{i=1}^{p}x_i^{(k)}\right)\left(\frac{1}{q'}\sum_{j'=1}^{q'}x_{j'}^{(k+m+n)}-\frac{1}{p'}\sum_{i'=1}^{p'}x_{i'}^{(k+m)}\right)\right]=\\
&\qquad E\left[\frac{1}{pp'}\sum_{i'=1}^{p'}\sum_{i=1}^{p}x_i^{(k)}x_{i'}^{(k+m)}+\frac{1}{qq'}\sum_{j'=1}^{q'}\sum_{j=1}^{q}x_j^{(k+n)}x_{j'}^{(k+m+n)}\right.\\
&\qquad\left.-\frac{1}{qp'}\sum_{i'=1}^{p'}\sum_{j=1}^{q}x_j^{(k+m)}x_{i'}^{(k+m)}-\frac{1}{pq'}\sum_{j'=1}^{q'}\sum_{i=1}^{p}x_i^{(k)}x_{j'}^{(k+m+n)}\right]
\end{aligned}
\tag{S48}
$$

As in the MSD derivation, we proceed in simplifying Eq. (S48) by taking the expectation value on the RHS in layers according to $E(\cdot)=\mathrm{E}_p\left(E_{t|p}\left(E_{x|t,p}(\cdot)\right)\right)$ and making use of the defining correlation function of FBM in Eq. (S1). Doing this and cancelling terms when appropriate gives:

$$E\left[\left(\frac{1}{q}\sum_{j=1}^{q}x_j^{(k+n)}-\frac{1}{p}\sum_{i=1}^{p}x_i^{(k)}\right)\left(\frac{1}{q'}\sum_{j'=1}^{q'}x_{j'}^{(k+m+n)}-\frac{1}{p'}\sum_{i'=1}^{p'}x_{i'}^{(k+m)}\right)\right]=$$
$$D^*E\left[-\frac{1}{pp'}\sum_{i'=1}^{p'}\sum_{i=1}^{p}\left|mt_E+t_{i'}^{(k+m)}-t_i^{(k)}\right|^\alpha-\frac{1}{qq'}\sum_{j'=1}^{q'}\sum_{j=1}^{q}\left|mt_E+t_{j'}^{(k+m+n)}-t_j^{(k+n)}\right|^\alpha\right.$$
$$\left.+\frac{1}{qp'}\sum_{i'=1}^{p'}\sum_{j=1}^{q}\left|(n-m)t_E+t_j^{(k+n)}-t_{i'}^{(k+m)}\right|^\alpha+\frac{1}{pq'}\sum_{j'=1}^{q'}\sum_{i=1}^{p}\left|(n+m)t_E+t_{j'}^{(k+m+n)}-t_i^{(k)}\right|^\alpha\right] \tag{S49}$$

In the case $m = n$, Eq. (S49) becomes:

$$E\left[\left(\frac{1}{q}\sum_{j=1}^{q}x_j^{(k+n)}-\frac{1}{p}\sum_{i=1}^{p}x_i^{(k)}\right)\left(\frac{1}{q'}\sum_{j'=1}^{q'}x_{j'}^{(k+m+n)}-\frac{1}{p'}\sum_{i'=1}^{p'}x_{i'}^{(k+m)}\right)\right]=$$
$$D^*E\left[\frac{1}{pq}\sum_{j=1}^{q}\sum_{i=1}^{p}\left|2nt_E+t_j^{(k+2n)}-t_i^{(k)}\right|^\alpha-\frac{2}{pq}\sum_{j=1}^{q}\sum_{i=1}^{p}\left|nt_E+t_j^{(k+n)}-t_i^{(k)}\right|^\alpha\right.$$
$$\left.+\frac{1}{p^2}\sum_{i=1}^{p}\left|t_j^{(k)}-t_i^{(k)}\right|^\alpha\right] \tag{S50}$$

We've already found expectation values of the forms on the RHS of Eq. (S50) in Eqs. (S13-S42). With this work already done, we conclude that in the case $m = n$ we have:

$$E\left[\left(\frac{1}{q}\sum_{j=1}^{q}x_j^{(k+n)}-\frac{1}{p}\sum_{i=1}^{p}x_i^{(k)}\right)\left(\frac{1}{q'}\sum_{j'=1}^{q'}x_{j'}^{(k+m+n)}-\frac{1}{p'}\sum_{i'=1}^{p'}x_{i'}^{(k+m)}\right)\right]=$$
$$\frac{D^*t_E^\alpha}{(\alpha+2)(\alpha+1)}\left[(2n+1)^{\alpha+2}+(2n-1)^{\alpha+2}-2(2n)^{\alpha+2}\right.$$
$$\left.-2(n+1)^{\alpha+2}-2(n-1)^{\alpha+2}+4n^{\alpha+2}+2-\frac{2}{\bar{p}}\right] \tag{S51}$$

Plugging into Eq. (S47) and using the established definitions of σ and $A(u)$ we have:

$$E\left[\hat{C}_v^{(n)}(m=n)\right]=\frac{1}{(nt_E)^2}\left[\frac{D^*t_E^\alpha\left(A(2n)-2A(n)+2\right)}{(\alpha+2)(\alpha+1)}-\sigma^2\right] \tag{S52}$$

which is the same as Eq. (21) in the main text.

Finally, we must return to Eq. (S49) and consider the case $m \neq 0$, n. This gives:

$$E\left[\left(\frac{1}{q}\sum_{j=1}^{q}x_j^{(k+n)}-\frac{1}{p}\sum_{i=1}^{p}x_i^{(k)}\right)\left(\frac{1}{q'}\sum_{j'=1}^{q'}x_{j'}^{(k+m+n)}-\frac{1}{p'}\sum_{i'=1}^{p'}x_{i'}^{(k+m)}\right)\right]=$$
$$D^{*}E\left[\frac{1}{pq}\sum_{j=1}^{q}\sum_{i=1}^{p}\left|(n+m)t_E+t_j^{(k+m+n)}-t_i^{(k)}\right|^{\alpha}+\frac{1}{pq}\sum_{j=1}^{q}\sum_{i=1}^{p}\left|\,|n-m|\,t_E+t_j^{(k+n)}-t_i^{(k+m)}\right|^{\alpha}\right.$$
$$\left.-\frac{2}{pq}\sum_{j=1}^{q}\sum_{i=1}^{p}\left|mt_E+t_j^{(k+m)}-t_i^{(k)}\right|^{\alpha}\right] \tag{S53}$$

Here we have made use of the fact that

$$\left|(n-m)t_E+t_j^{(k+n)}-t_i^{(k+m)}\right|=\begin{cases}(n-m)t_E+t_j^{(k+n)}-t_i^{(k+m)} & ,n>m\\ (m-n)t_E+t_i^{(k+m)}-t_j^{(k+n)} & ,\mathrm{m}>\mathrm{n}\end{cases} \tag{S54}$$

and that $\left(t_j^{(k+n)}-t_i^{(k+m)}\right)\sim\left(t_i^{(k+m)}-t_j^{(k+n)}\right)$, and thus

$$E\left[\frac{1}{pq}\sum_{j=1}^{q}\sum_{i=1}^{p}\left|(n-m)t_E+t_j^{(k+n)}-t_i^{(k+m)}\right|^{\alpha}\right]=E\left[\frac{1}{pq}\sum_{j=1}^{q}\sum_{i=1}^{p}\left|\,|n-m|\,t_E+t_j^{(k+n)}-t_i^{(k+m)}\right|^{\alpha}\right] \tag{S55}$$

As before, the remaining expectation values in Eq. (S53) can be evaluated by noting analogies with the expressions derived in the MSD section to finally give (when $m \neq 0$, n):

$$E\left[\left(\frac{1}{q}\sum_{j=1}^{q}x_j^{(k+n)}-\frac{1}{p}\sum_{i=1}^{p}x_i^{(k)}\right)\left(\frac{1}{q'}\sum_{j'=1}^{q'}x_{j'}^{(k+m+n)}-\frac{1}{p'}\sum_{i'=1}^{p'}x_{i'}^{(k+m)}\right)\right]=$$
$$\frac{D^{*}t_E^{\alpha}}{(\alpha+2)(\alpha+1)}\left[(n+m+1)^{\alpha+2}+(n+m-1)^{\alpha+2}-2(n+m)^{\alpha+2}\right.$$
$$\left.\left(|n-m|+1\right)^{\alpha+2}+\left(|n-m|-1\right)^{\alpha+2}-2|n-m|^{\alpha+2}-2(m+1)^{\alpha+2}-2(m-1)^{\alpha+2}+4m^{\alpha+2}\right] \tag{S56}$$

Plugging into Eq. (S47) and using the established definition $A(u)$ we have:

$$E\left[\hat{C}_v^{(n)}(m\neq 0,n)\right]=\frac{D^{*}t_E^{\alpha}\left[A(n+m)-2A(m)+A\left(|\,n-m\,|\right)\right]}{(nt_E)^2(\alpha+2)(\alpha+1)} \tag{S57}$$

which is the same as Eq. (22) in the main text. Thus we have derived the expected value of the VAC in all cases.

SUPPLEMENTAL FIGURES

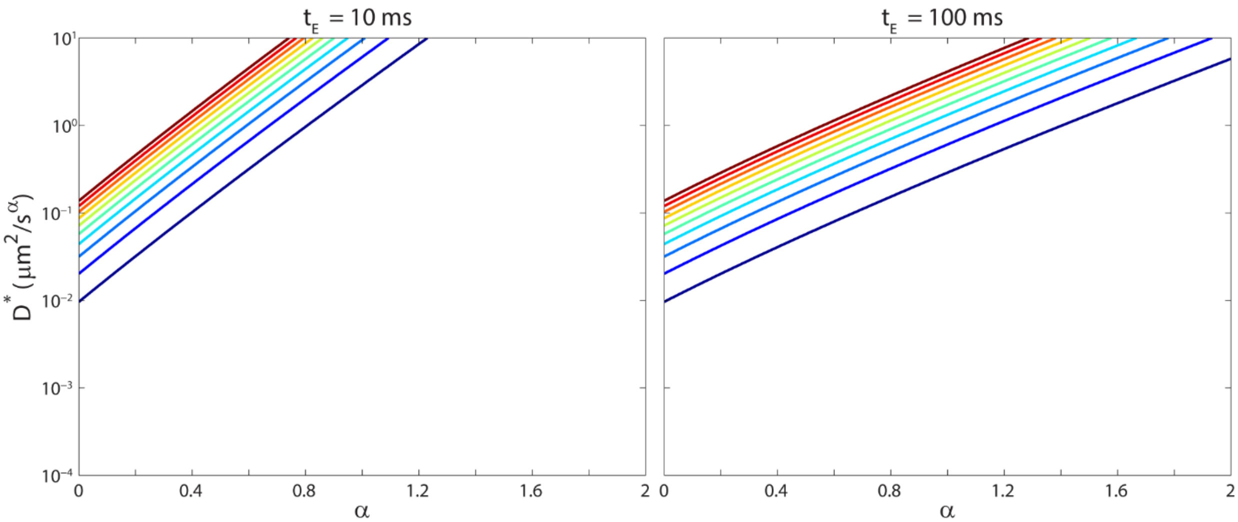

FIG S1 Static error inflation ratio, $\frac{\sigma}{\sigma_0}$, as defined in Eq. (7) of the main text. We show contour plots for two different t_E values: 10 ms and 100 ms. Contour line values range from 1.1 (dark blue) to 2.0 (dark red), spaced by 0.1. In both plots, the ratio continues to increase beyond 2.0 toward the top left corner. In fact, as α → 0, $\frac{\sigma}{\sigma_0} \to \sqrt{1+\frac{D^*}{s_0^2}}$, which increases without bound as D^* increases.

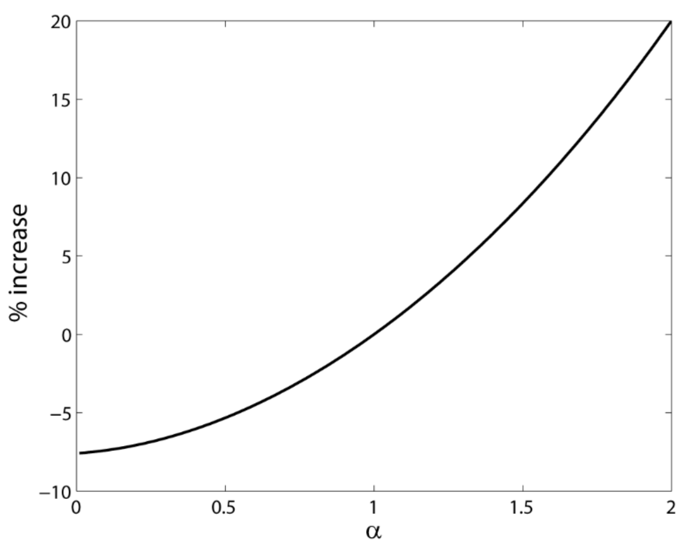

FIG S2 Percent increase in MSD from inclusion of terms beyond n^{α}. Here we consider the point n = 1 in the limit σ = 0 (or equivalently, when the static error has been carefully measured and removed from the MSD). The percent increase is defined via:

$$\%\text{increase} = \frac{\left\{ \frac{2D^* t_E^{\alpha}}{(\alpha+2)(\alpha+1)} \left[(n+1)^{\alpha+2} + (n-1)^{\alpha+2} - 2n^{\alpha+2} - 2 \right] - \left[2D^*(nt_E)^{\alpha} - \frac{4D^* t_E^{\alpha}}{(\alpha+2)(\alpha+1)} \right] \right\} \times 100\%}{2D^*(nt_E)^{\alpha} - \frac{4D^* t_E^{\alpha}}{(\alpha+2)(\alpha+1)}}$$

For n = 1 this simplifies to:

$$\%\text{increase} = \frac{\left[2^{\alpha+2} - 2 - (\alpha+2)(\alpha+1) \right] \times 100\%}{(\alpha+2)(\alpha+1) - 2}$$

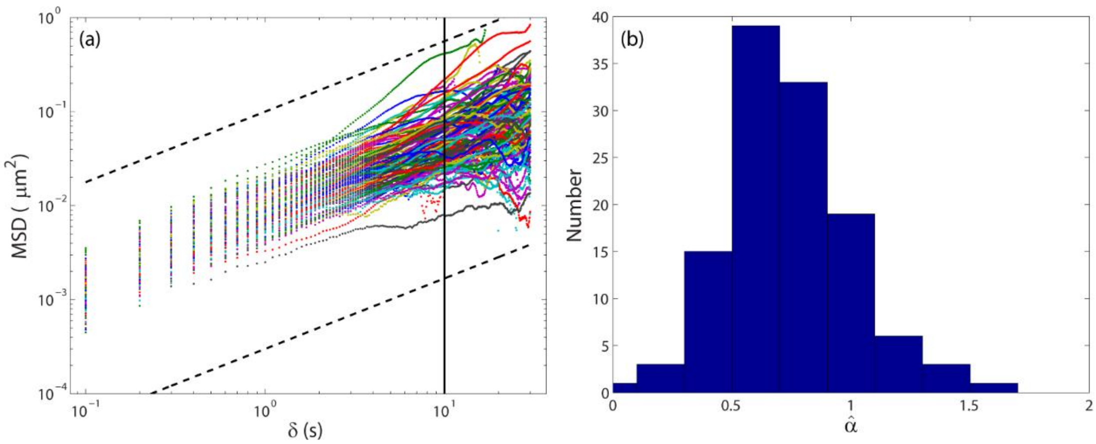

FIG S3 (a) All 120 individual time-averaged MSDs from ensemble of experimental chromosomal loci data. Dashed black lines have a slope corresponding to α = 0.75 and are meant to guide the eye. The solid black vertical line indicated the cutoff time at which we limited our mean MSD analysis. (b) Histogram of estimated α parameters as determined by individually fitting each of the 120 MSDs shown in (a) up to the first 100 time lags. The distribution has mean 0.74 and standard deviation 0.26.

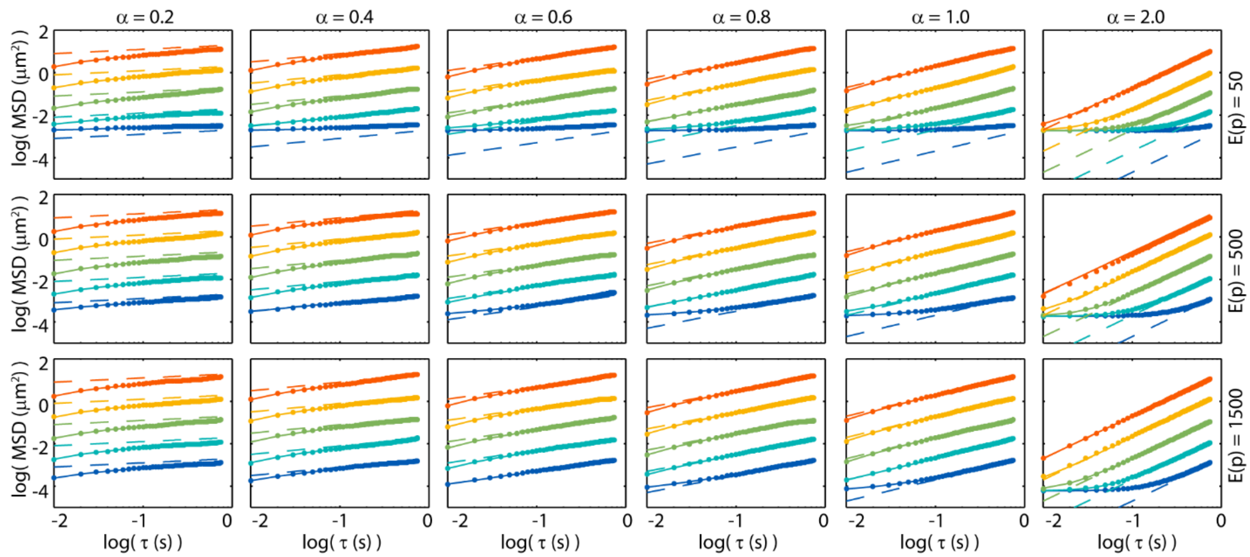

FIG S4 MSDs from same 1D simulation described in text for various α and photon counts. $D^* = 10^{-3}$ (blue), 10^{-2} (cyan), 10^{-1} (green), 10^{0} (yellow), and 10^{1} (red). The axis labels refer to the base-10 logarithm.

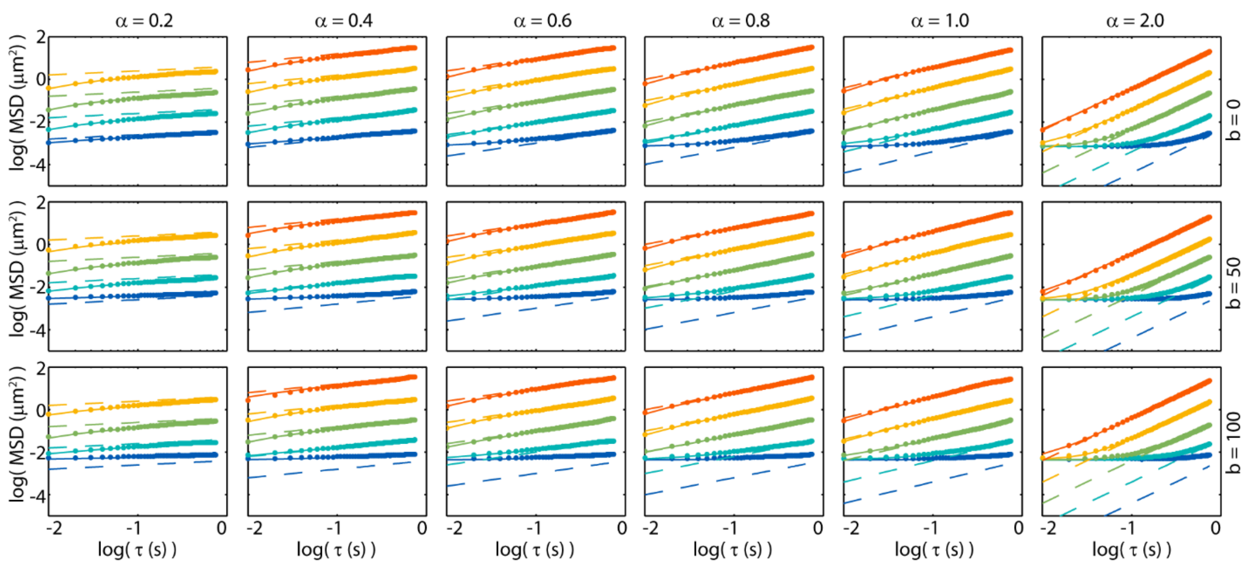

FIG S5 MSDs from same 2D simulation set as in Fig. 2(b) in the main text for various α and background levels, extended to include all of $\alpha \in \{0.2, 0.4, 0.6, 0.8, 1.0, 2.0\}$. Colors correspond to same D^* as in Figs. S1 and 2(b). The axis labels refer to the base-10 logarithm.

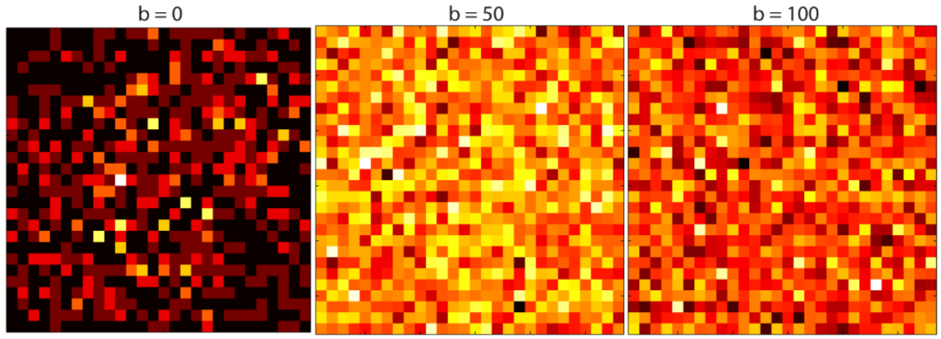

FIG S6 Examples of images of a particle with α = 0.2, D^* = 10, and $\bar{p} = 500$ for various b. The lack of a detectable cluster of signal photons above the noise in all cases is justification for omitting these cases from Fig. 2(b) and Fig. S4.

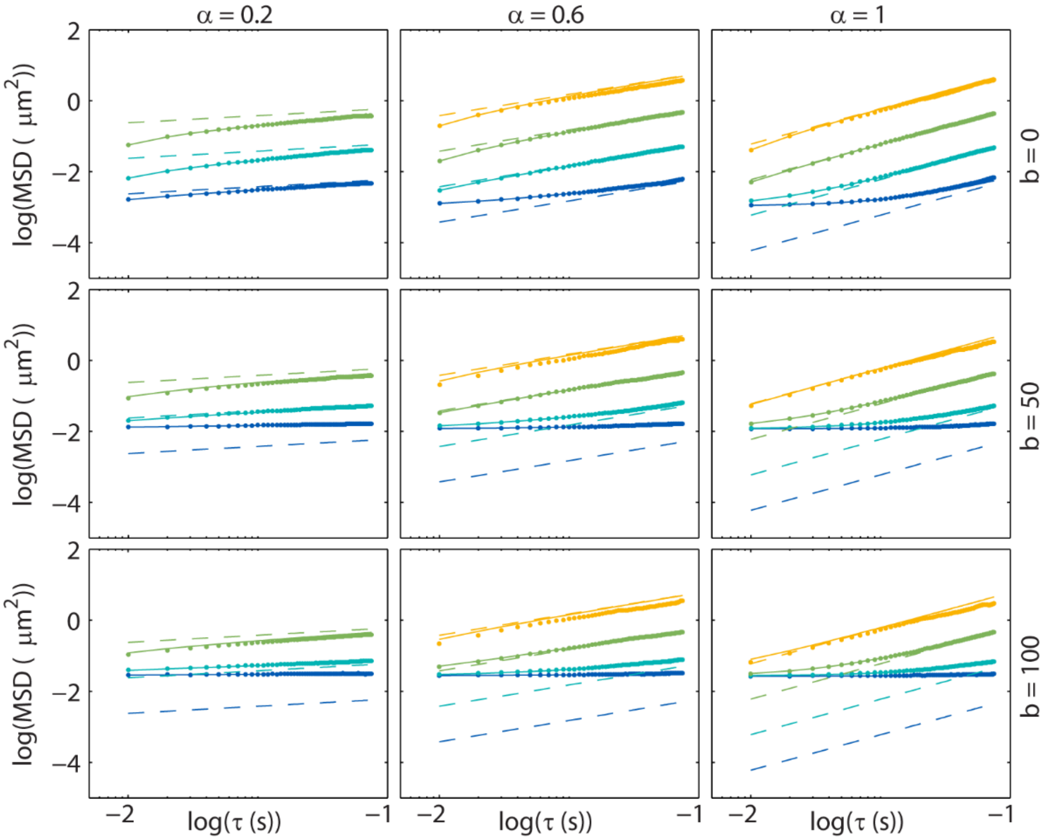

FIG S7 Simulation results for 3D tracking using the DH-PSF with $\bar{p} = 500$. The axis labels refer to the base-10 logarithm. Colors correspond to same D^* as in Figs. S1 and 2(b). 3D trajectories were produced by using the same circulant embedding approach, but with the addition of a z dimension. Each image was produced by approximating the DH-PSF as two 2D Gaussian with amplitude, spacing, and relative angle determined by calibration data of the true PSF, then adding the appropriate background and noise. This Gaussian approximation was used in the interest of time, though using the true PSF is not expected to change the major results and behavior. The noisy images were then fit to the sum of two Gaussian functions and the x, y, and z positions were estimated and compared to the true input positions. Note that here we do not have an explicit model for σ, and instead we can compute it directly since we know the true underlying positions. In a real experiment these positions are not known and so σ can be treated as a free parameter, as shown in the main text. Note that we do not display any data for $D^* = 10$, nor for ($D^* = 1$, $\alpha = 0.2$). This is because the images in these cases would often be thrown away and not fit in the same way that we threw away the case of ($D^* = 10$, $\alpha = 0.2$) in the 2D tracking case. The more liberal threshold in this 3D case is justified by the fact that the DH-PSF spreads the same photons now over >2 times the area, and so the image deteriorates more quickly. Our choice of threshold is supported by the example images shown in Fig. S7.

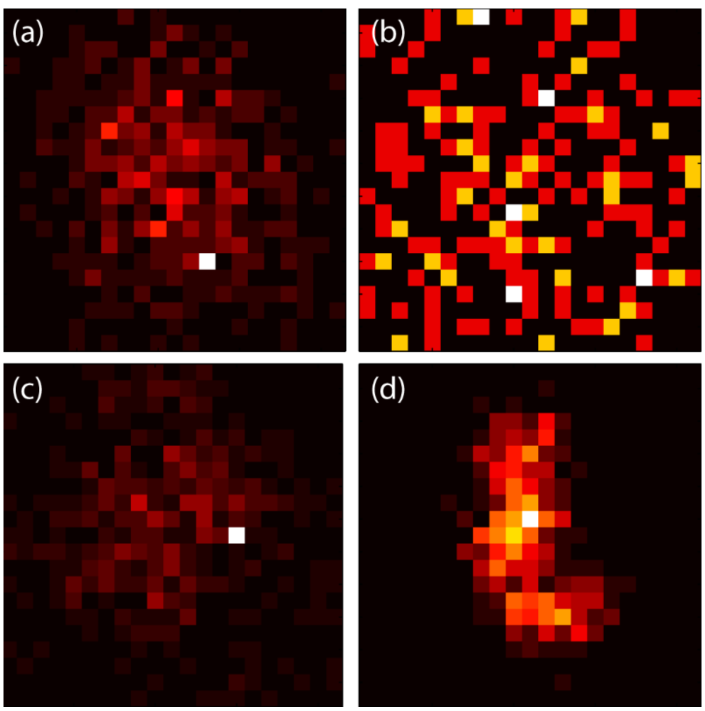

FIG S8 Example simulated images of DH-PSF justifying omission from Fig. S6 for the cases of (a) ($D^* = 1$, $\alpha = 0.2$), (b) ($D^* = 10$, $\alpha = 0.2$), (c) ($D^* = 10$, $\alpha = 0.6$), and (d) ($D^* = 10$, $\alpha = 1.0$). The two lobes of the DH-PSF are not easily discernable and so these images would likely be discarded in a real experiment. Note that the $b = 0$ in each of these images and so they only get worse for nonzero b. Pixel size = 160 nm.

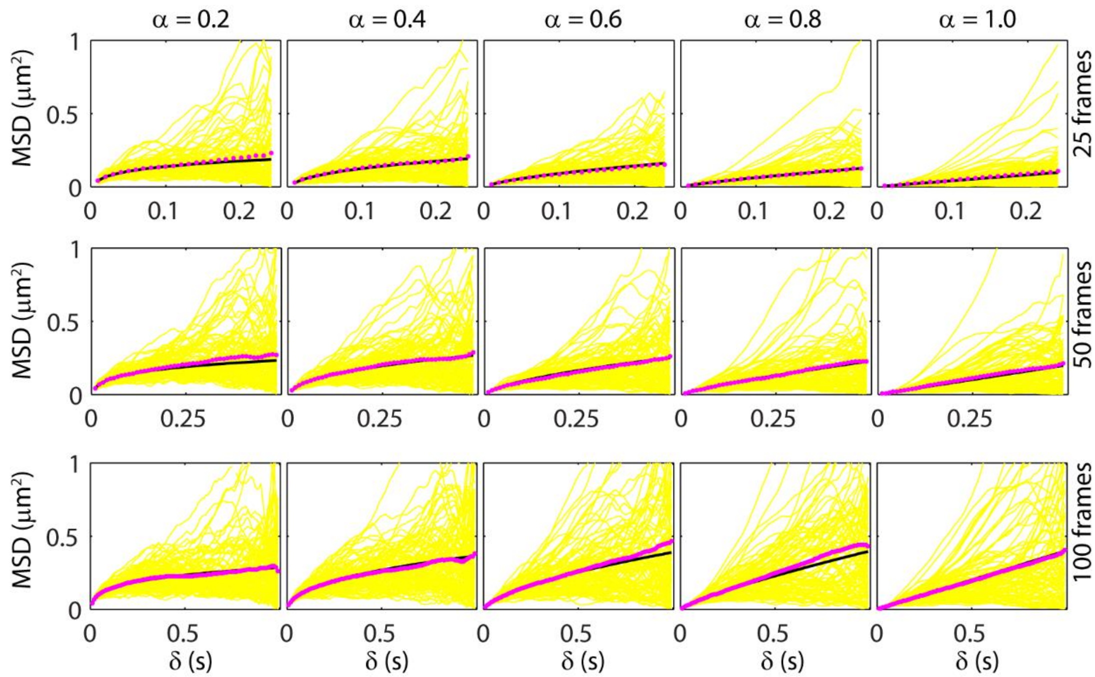

FIG S9 Simulated MSDs plotted in linear space. Each panel corresponds to α at the top of the column and the number of frames per each track at the right of the row. For these simulated data t_E = 10 ms, D^* = 0.1 μm^2/s$^\alpha$, $\bar{p} = 500$, and b = 50. Yellow curves are each of the 100 time-averaged MSDs computed for each individual track, and the magenta lines are the ensemble mean of this distribution of yellow curves. The black line corresponds to the expected value according to Eq. (8), so when it is obscured the simulated data match the theory very well. Discrepancies at longer times in some of the panels are indicative of errors due to finite sampling.

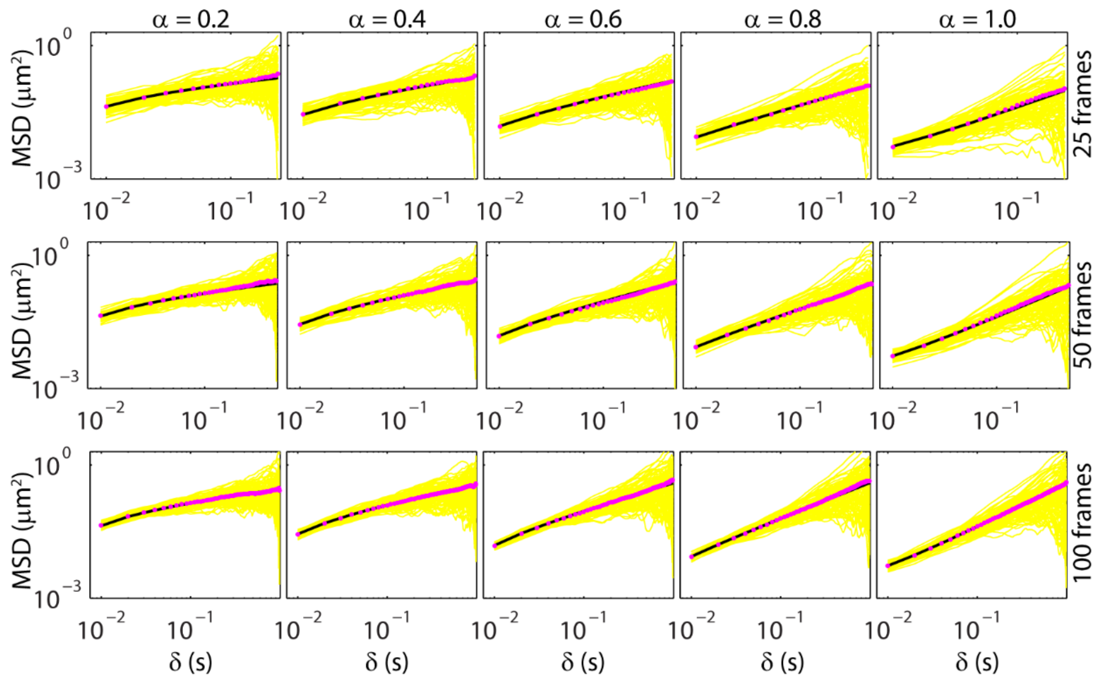

FIG S10 Same data and explanation as in Fig. S8 except plotted on a log-log scale.

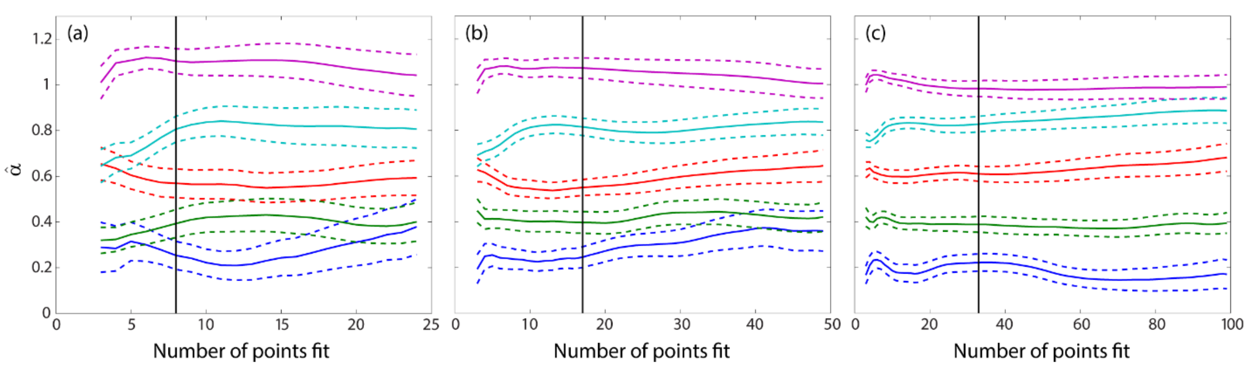

FIG S11 Estimated α from same simulated data as in Fig. S8 and S9, for various numbers of points fit in the MSD. Tracks were truncated at (a) 25 frames, (b) 50 frames, or (c) 100 frames. Colored lines (from bottom to top) correspond to simulated α of 0.2 (blue), 0.4 (green), 0.6 (red), 0.8 (cyan) and 1.0 (magenta). Solid lines are mean estimates of α from 100 bootstrapped samples of the 100-track ensembles. Dashed lines are the standard deviations. Vertical black lines in each panel mark our heuristically determined rule-of-thumb for fitting the first third of the points of the MSD.

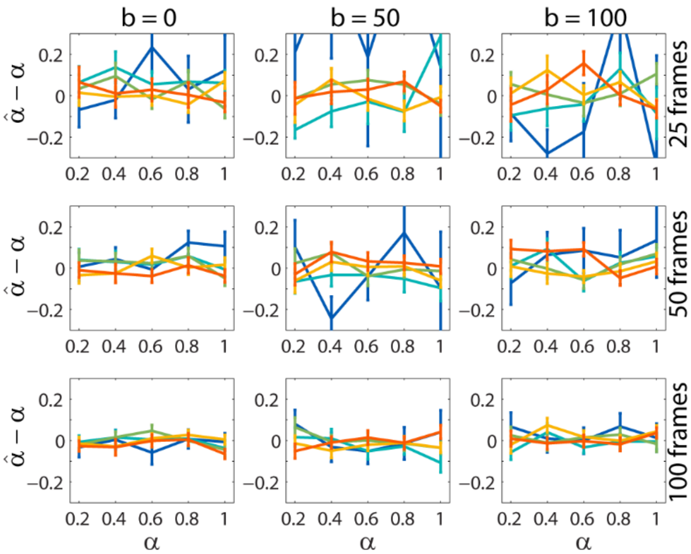

FIG S12 Bias and standard error (error bars) in estimated α for 2D simulated data, as determined by fitting Eq. (8) with free parameters α, D^*, and σ using the heuristically determined rule-of-thumb of fitting the first third of the points of the MSD. Here we fixed t_E = 10 ms and $\bar{p} = 500$. Each panel corresponds to the background level indicated at the top of the column and the individual track length at the right of the row. Error bars were computed by finding the S. E. M. from 100 bootstrapped samples of the 100-track ensemble. The colors in each panel indicated the simulated D* in accordance with Figs. 2(b), S3, S4, and S6.

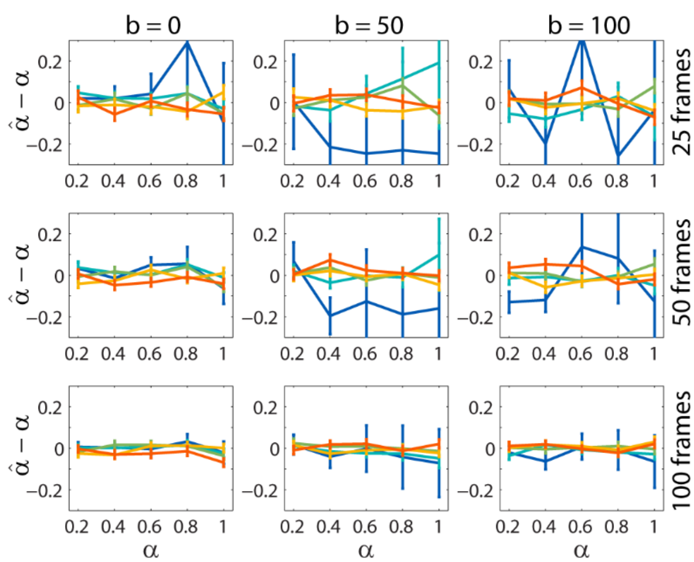

FIG S13 Same data and explanation as in Fig. S11 except that we measured σ and removed the offset of $2\sigma^2$ before fitting to the resulting modified version of Eq. (8) with only two free parameters D^* and α.

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