

## Electronic Supplementary Information

### 1 Materials and Methods

#### 1.1 Computation

All numerical calculations were performed using MATLAB. We used a standard particle tracking algorithm<sup>2</sup>. When searching for the maximum of the log-likelihood function  $L_\delta(\alpha)$ , we used built-in optimization functions *fminsearch* and *particleswarm*. Note that these algorithms search for the minimum, not maximum, of a function of several variables, so we applied them to  $-L_\delta(\alpha)$ . We have found fairly comparable performances from these two algorithms, but we point out their differences. Function *fminsearch* requires a guess input about the location of the minimum. It is fast, but may have the tendency of ending far from the global minimum, so that it is highly recommended, in our view, to try many different guess inputs and check for consistency. Function *particleswarm* is a random algorithm, and will only look for a minimum within an interval defined by the user. It is slower, but might be more robust. More details about the algorithms underlying *fminsearch* and *particleswarm* are available in the MATLAB documentation.

#### 1.2 Experiments

We used Dynabeads M-280 Streptavidin particles (2.8  $\mu\text{m}$  diameter) as paramagnetic spheres. The spheres were resuspended in deionized water with 0.5% bovine serum albumin to prevent sticking to the glass surfaces. A droplet of the resulting solutions was deposited in a sealed, thin chamber (Secure-Seal spacer from Life technologies, 9 mm diameter and 120  $\mu\text{m}$  thickness) in order to prevent any flow by turbulence or evaporation. Observation was realized using an inverted microscope (Nikon TE-2000), equipped with a N.A. 1.4 100 $\times$  oil immersion objective lens. Movies were recorded using a fast camera (Photron Fastcam 1024PCI).

### 2 Mathematical support to maximum likelihood analysis

#### 2.1 Random variable with fixed mean and standard deviation

Let us consider  $N$  results of a random experiment,  $\{x_1, \dots, x_N\}$ . We assume that the distribution from which these points are drawn is a normal (or Gaussian) distribution  $\mathcal{N}_{\mu, \sigma}$  of mean  $\mu$  and standard deviation  $\sigma$ :

$$\mathcal{N}_{\mu, \sigma}(x) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right), \quad (1)$$

where  $\mu$  and  $\sigma$  are not known *a priori*. The goal of the maximum likelihood analysis is to estimate  $\mu$  and  $\sigma$ . We can define a function  $\mathcal{L}_{\mu', \sigma'}$  of the set of values  $\{x_1, \dots, x_N\}$  and parameters  $\mu'$  and  $\sigma'$ , called log-likelihood function, as:

$$\mathcal{L}_{\mu', \sigma'}(\{x_1, \dots, x_N\}) = \frac{1}{N} \sum_{i=1}^N \ln(\mathcal{N}_{\mu', \sigma'}(x_i)). \quad (2)$$

In the limit of a large dataset ( $N \rightarrow \infty$ ),  $\mathcal{L}_{\mu', \sigma'}(\{x_1, \dots, x_N\})$  tends

towards  $\mathcal{L}_{\mu', \sigma'}^\infty$ , where:

$$\mathcal{L}_{\mu', \sigma'}^\infty = \int_{-\infty}^{+\infty} \mathcal{N}_{\mu, \sigma}(x) \times \ln(\mathcal{N}_{\mu', \sigma'}(x)) dx \quad (3)$$

$$= \ln\left(\frac{1}{\sqrt{2\pi\sigma'^2}}\right) - \frac{(\mu - \mu')^2 + \sigma'^2}{2\sigma'^2}. \quad (4)$$

Consequently,  $\mathcal{L}_{\mu', \sigma'}^\infty$  is maximum when both  $\mu = \mu'$  and  $\sigma = \sigma'$ . Therefore, the set of values  $\{\mu', \sigma'\}$  which maximizes  $\mathcal{L}_{\mu', \sigma'}^\infty$  corresponds to the actual mean and standard deviation of the distribution from which the set  $\{x_1, \dots, x_N\}$  comes from.

#### 2.2 Functional mean and standard deviation

We now consider the case of  $N$  uncorrelated, two-dimensional random variables  $\{(x_i, \delta_i)_{i=1..N}\}$ . The  $x_i$  values are drawn from some distribution  $\rho(x)$ , and the  $\delta_i$  values are drawn from a normal distribution of mean and standard deviation depending on their prior,  $x_i$ , through two functions  $\hat{\mu}(x)$  and  $\hat{\sigma}(x)$ , respectively. Given any two functions  $\hat{\mu}'$  and  $\hat{\sigma}'$  we can define the log-likelihood function as:

$$\mathcal{L}_{\hat{\mu}', \hat{\sigma}'}(\{(x_i, \delta_i)_{i=1..N}\}) = \frac{1}{N} \sum_{i=1}^N \ln\left(\mathcal{N}_{\hat{\mu}'(x_i), \hat{\sigma}'(x_i)}(\delta_i)\right), \quad (5)$$

which in the  $N \rightarrow \infty$  limit converges towards:

$$\mathcal{L}_{\hat{\mu}', \hat{\sigma}'}^\infty = \iint \mathcal{N}_{\hat{\mu}(x), \hat{\sigma}(x)}(\delta) \times \ln\left(\mathcal{N}_{\hat{\mu}'(x), \hat{\sigma}'(x)}(\delta)\right) \rho(x) dx d\delta. \quad (6)$$

**Theorem 1** *The log-likelihood  $\mathcal{L}_{\hat{\mu}', \hat{\sigma}'}^\infty$  is maximum when  $\hat{\mu}'(x) = \hat{\mu}(x)$  and  $\hat{\sigma}'(x) = \hat{\sigma}(x)$ , for all  $x$ .*

Indeed, from the previous section, for any two functions  $\hat{\mu}'$ ,  $\hat{\sigma}'$  we have  $\forall x$ :

$$\int \mathcal{N}_{\hat{\mu}(x), \hat{\sigma}(x)}(\delta) \times \ln\left(\mathcal{N}_{\hat{\mu}'(x), \hat{\sigma}'(x)}(\delta)\right) d\delta \quad (7)$$

$$\leq \int \mathcal{N}_{\hat{\mu}(x), \hat{\sigma}(x)}(\delta) \times \ln\left(\mathcal{N}_{\hat{\mu}(x), \hat{\sigma}(x)}(\delta)\right) d\delta. \quad (8)$$

Therefore by multiplication by  $\rho(x)$  (positive) and integration over the  $x$ -domain we get immediately that  $\mathcal{L}_{\hat{\mu}', \hat{\sigma}'}^\infty \leq \mathcal{L}_{\hat{\mu}, \hat{\sigma}}^\infty$ .