

## MIT Open Access Articles

*On the correction of errors in some  
multiple particle tracking experiments*

The MIT Faculty has made this article openly available. **Please share** how this access benefits you. Your story matters.

**Citation:** Rowlands, Christopher J., and Peter T. C. So. "On the Correction of Errors in Some Multiple Particle Tracking Experiments." Applied Physics Letters 102, no. 2 (January 14, 2013): 021913.

**As Published:** <http://dx.doi.org/10.1063/1.4776738>

**Publisher:** AIP Publishing

**Persistent URL:** <http://hdl.handle.net/1721.1/120340>

**Version:** Final published version: final published article, as it appeared in a journal, conference proceedings, or other formally published context

**Terms of Use:** Article is made available in accordance with the publisher's policy and may be subject to US copyright law. Please refer to the publisher's site for terms of use.



## On the correction of errors in some multiple particle tracking experiments

Christopher J. Rowlands<sup>1,a)</sup> and Peter T. C. So<sup>2</sup>

<sup>1</sup>*Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA*

<sup>2</sup>*Departments of Mechanical and Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA*

(Received 24 September 2012; accepted 3 January 2013; published online 17 January 2013)

A common source of error in particle tracking experiments is identified, and a theoretical model for the magnitude of the error is offered. In many cases, the error is small, but in systems where only a few particles are being tracked, the measured mean squared displacement can be up to 50% smaller than the actual value. The theoretical model predictions are confirmed using numerical simulations and experimental observations of polystyrene microspheres in water. © 2013 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4776738>]

Multiple particle tracking is commonly used to obtain information about the local rheology in microscale systems, by using a video camera to track easily identified particles as they move within a sample.<sup>1–3</sup> Early developments in single-particle tracking were made by Mason *et al.*,<sup>4</sup> shortly followed by the development of two-particle tracking.<sup>5</sup> Many authors have also provided additional insight into possible sources of error in multiple particle tracking, from statistical sampling biases<sup>6</sup> to the validity of the Generalized Stokes-Einstein Relation (GSER),<sup>7</sup> the effects of noise and exposure time<sup>8</sup> and the effects of the chemical interaction between the particles and their environment.<sup>9</sup>

This paper describes a subtle error, which may be present in a number of measurements in the literature. This error occurs because the displacements commonly measured for particle tracking are very small; on the order of nanometers. This is below the diffraction limit of light, so the spatial resolution is achieved through the fitting of the intensity distribution of the image of the particle to a known mathematical function, such as a Gaussian function, or by calculating the centroid of the intensity distribution. Despite the use of vibrationally damped optical tables and carefully constructed experiments, vibration from the local environment can often overwhelm the tiny displacements being measured. Consequently, it is common to utilize “de-drifting,” or the subtraction of the mean displacement vector of the measured particles, recognising that external vibrational influences should cause the same apparent motion in every particle. Unfortunately, this process can lead to errors in the mean-squared displacement (MSD) of the individual particles, as some motion that is entirely independent of external vibration is erroneously subtracted from all the particles. This error is present in the scientific literature,<sup>10</sup> as well as the popular interactive data language (IDL) particle tracking code of Crocker *et al.*,<sup>5</sup> which is used in several recent publications,<sup>11–13</sup> as well as the MATLAB implementation of Crocker *et al.*'s code by the Kilfoil laboratory at the University of Massachusetts, Amherst. Many other particle-tracking codes used in the scientific literature, such as Imaris (Bitplane, St.

Paul, Minneapolis, USA) and Metamorph (Universal Imaging Corporation, Downingtown, Pennsylvania, USA) are proprietary, and consequently it has not been possible to assess whether results obtained using these software packages are subject to this type of error.

Corrigan and Donald offer a method for de-drifting in a two-particle tracking experiment, which does not involve taking the mean of the particle positions; rather it places an additional constraint that the correlations between particles should decay as  $1/r$ , where  $r$  is the distance between particles.<sup>14</sup> Since this method can only be used to correct two-particle tracking experiments, it cannot be directly compared with the correction described in this paper, hence is included for completeness only.

The multiple particle tracking experiment is modelled as  $n$  randomly distributed particles, which undergo a random walk independently of each other; at each time-step, a different random vector is added onto each particle to determine its new location. This random vector is drawn from a normal distribution with a mean of zero in all dimensions and a variance of  $\alpha$  in all dimensions. In addition, external vibration is modelled as a separate random walk with a mean of zero and a variance of  $\beta$ . The location of any particle at time  $(t + \tau)$ , where  $t$  is the time and  $\tau$  is the magnitude of the time-step of the model or measurement, is, therefore, given by the following expression:

$$x_{i,(t+\tau)} = x_{i,t} + Y_i + Z, \quad (1)$$

where  $x_{i,t}$  indicates the position of particle  $i$  at time  $t$ .  $Z \sim \mathcal{N}(0, \beta)$  is a constant for all particles, and  $Y_i \sim \mathcal{N}(0, \alpha)$  is different for each particle;  $\mathcal{N}(\mu, \sigma^2)$  describes a normal distribution with a mean of  $\mu$  and a variance of  $\sigma^2$ . Note that, in this context, “ $\sim$ ” means “is drawn from” as opposed to “is approximately equal to.”

In order to correct for the influence of the  $Z$  term, it is common to subtract the mean displacement of all the measured particles, since for an infinite number of measured particles,

$$\frac{\sum_{i=1}^n (x_{i,(t+\tau)} - x_{i,t})}{n} = Z, \quad (2)$$

<sup>a)</sup>Electronic mail: [chrisrow@mit.edu](mailto:chrisrow@mit.edu).

because  $(\sum_{i=1}^n Y_i/n) = 0$ . For a finite number of particles however, this does not necessarily hold true. By subtracting the mean, a proportion of the independent motion of each particle will be subtracted as well. The magnitude of this subtracted motion can be expressed as the absolute value of the mean displacement of all of the particles

$$\hat{Z} = \sqrt{\frac{(\sum_{i=1}^n Y_i)^2}{n^2}}, \quad (3)$$

where  $\hat{Z}$  is a constant that varies for each value of  $t$ . Since the sum of a series of samples from a normal distribution is normally distributed,<sup>15</sup> such that  $\sum_{i=1}^n Y_i \sim \mathcal{N}(0, n\alpha)$ ,

$$E[\hat{Z}] = E\left[\sqrt{\frac{Y'^2}{n^2}}\right], \quad (4)$$

where  $E$  is the expectation operator and  $Y' \sim \mathcal{N}(0, n\alpha)$ . The square of a normally distributed variable is distributed according to the chi-squared distribution, in accordance with the relation  $\frac{\sum_{i=1}^k X_i^2}{\sigma^2} \sim \chi_k^2$ , where  $k$  is the degrees of freedom (one, in this case) and  $X_i$  is simply a random variable drawn from a normal distribution  $\mathcal{N}(0, \sigma^2)$ . Since the expectation of a chi-squared distributed variable is given by its degrees of freedom,

$$E[\hat{Z}] = \sqrt{\frac{\alpha n}{n^2}} = \sqrt{\frac{\alpha}{n}}. \quad (5)$$

With this result in hand, it is necessary to turn to the ‘‘measurement’’ part of the experiment. The mean-squared displacement,  $W$ , in the absence of any external vibration is determined as follows:

$$W = \frac{\sum_{i=1}^n (x_{i,(t+\tau)} - x_{i,t})^2}{n}. \quad (6)$$

Invoking the expectation of a chi-squared-distributed random variable once again, it is possible to assert that, when averaged over infinite  $t$ ,  $W = \alpha$ . But, as we have determined above, a certain fraction of the observed mean-squared displacement will be subtracted, resulting in the actual measured mean-squared displacement being given by

$$W_{measured} = \alpha - E[\hat{Z}]^2 = \alpha \left(1 - \frac{1}{n}\right). \quad (7)$$

Obtaining the correct value of  $\alpha$  is, therefore, only a matter of calculating  $\frac{nW_{measured}}{(n-1)}$ .

All the code for the numerical simulations was written using MATLAB 2011b. Two simulations were performed to demonstrate the validity of the theoretical predictions; both simulations had a total duration of 100 000 frames. The simulations take  $n$  randomly distributed particles, and at each time-step, add a random 2D vector to them. This vector is drawn from a two-dimensional normal distribution and has an arbitrarily selected standard deviation of  $1 \times 10^{-5}$ . An

external vibration is simulated as another random walk with a standard deviation of  $1 \times 10^{-4}$ , and the external vibration is added to particle’s location for each time-step. The MSD for a given time-step is determined by subtracting the position at time  $t$  from the position at time  $t + \tau$  where  $\tau$  is the time-step in frames. This is repeated for all possible combinations of frames, and the result averaged to produce the MSD for a given  $\tau$ .

The results, given in Figure 1, are in excellent agreement with the theoretical model. The increased noise at higher time steps is inherent to the manner in which the MSD is calculated and not due to attempts to correct for external vibration. It occurs because for higher time steps, there are fewer independent measurements to average over; in the case of a time-step of 50 000 frames, for example, there are only two independent measurements available (i.e., the displacement between the 1st and 50 000th point, and between the 50 001st and 100 000th point). All other measurements are at least partially correlated with these two.

In order to confirm that the assumptions in the numerical simulations and theoretical model accurately reflect reality, experiments were performed on a particle tracking system using particles suspended in deionised water.  $2 \mu\text{m}$  diameter fluorescent polystyrene beads (F-8888, Invitrogen) were suspended in distilled water with a final volume fraction of beads to water of 0.04%. Several drops of this suspension were placed on a petri dish with a glass coverslip bottom (P35G-1.5-14-C, MatTek corporation). The petri dish was sealed using paraffin film (Parafilm, Pechiney Plastic Packaging Company) to reduce evaporation. The beads were imaged under brightfield illumination using an inverted microscope (Axiovert 100, Carl Zeiss Microscopy, LLC) with a  $40\times$  oil-immersion objective (Zeiss Fluor  $40 \times 1.30\text{NA}$ ) and high-frame-rate camera with a resolution of  $640 \times 480$  (GE680, Allied Vision Technologies). A mean of approximately 38 beads were in focus at any one time. Feature extraction and tracking were performed using existing MATLAB code written by the Kilfoil laboratory at the University of Massachusetts, Amherst, but calculation of the MSD and subtraction of the average motion of all the particles was performed using custom MATLAB code. Characterization of the system was performed using the same  $2 \mu\text{m}$  beads; several drops of the same 0.04% v/v suspension were placed onto a glass microscope slide, allowed to dry, and then coated with cyanoacrylate (Krazy Glue, Elmer’s Products, Inc.) and a glass coverslip. The MSD was found to be  $1.0735 \times 10^{-17}\text{m}^2$  per time step, with de-drifting applied, and with a time step of 5.88 ms. 109 particles were tracked (the maximum available in the field of view) in order to minimise the error from de-drifting.

In order to demonstrate the effect of using fewer particles to calculate the MSD, for each pair of adjacent frames in the captured video, the particles present in both frames were determined. Datasets containing between 2 particles and the maximum number of common particles were created. For each dataset, the mean displacement vector was subtracted from the displacement vector for each particle, and the mean-squared-displacement calculated for that dataset. These MSDs were averaged over all frames and for all particle numbers. The results can be seen in Figure 2.

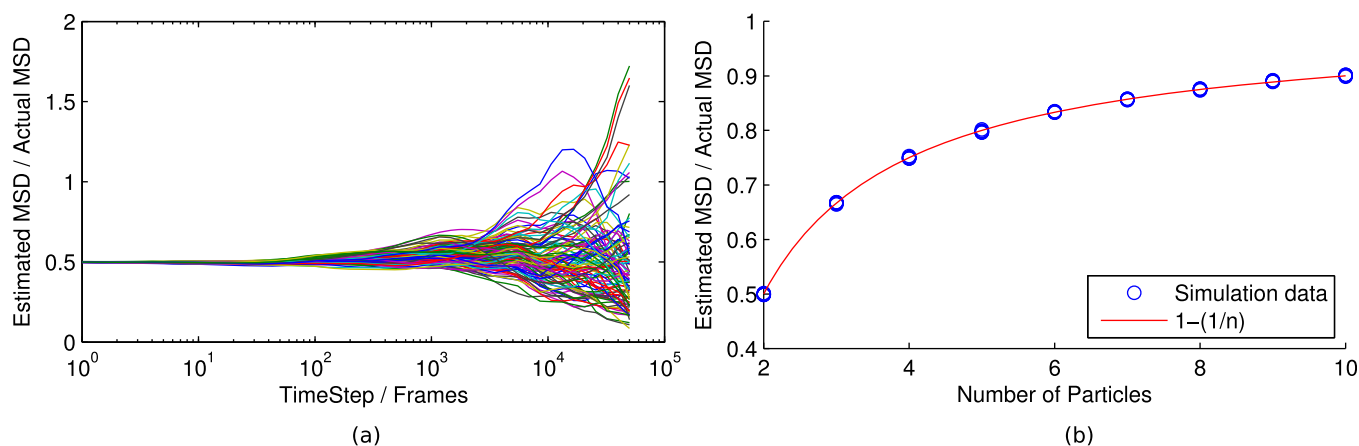


FIG. 1. Confirmation of theoretical predictions using numerical simulations. (a) The measured MSD (normalized to the actual MSD) as a function of time step for 100 separate simulations. Each simulation has two tracked particles, so the MSD is predicted to be half the actual value, as demonstrated by the simulations. (b) Analysing the effect of tracking more particles. For each  $n$ , eight different simulations are plotted, and for each simulation, the mean value of the MSD for a time-step of 1 is plotted. As a guide to the eye, the line corresponding to  $1 - \frac{1}{n}$  is plotted, showing excellent agreement between the theoretical prediction and the numerical result.

It is possible to demonstrate the improvement in measurement accuracy by attempting to measure the viscosity of samples with well-characterized literature values. The samples measured were 0 mM, 2 mM, and 4 mM solutions of D-glucose (Mallinckrodt) in distilled water, with  $2\ \mu\text{m}$  fluorescent beads (F-8888, Invitrogen) added, to yield a solution with 0.04% solids as before. A sample chamber was constructed by gluing a cell perfusion gasket (C-18139, Molecular Probes) to a microscope slide, filling each well with a different solution, and placing a coverslip on top. The samples were left inverted before measurement, and imaged using the same microscope as previously by inverting the sample chamber, focusing  $30\ \mu\text{m}$  into the sample volume to minimise the influence of the chamber walls, and waiting for the beads to enter the field of view. Once a sufficient number entered the field of view, video capture was started. Approximately 5000 frames were captured for each sample, at 170 frames per second.

The ambient temperature was measured to be 286 K using an alcohol thermometer. By calculating the diffusion coefficient and using the Stokes-Einstein relationship with the known radius of the bead, it was possible to calculate the

viscosity of the sample. The diffusion coefficient was calculated by fitting a plot of the number of tracked particles vs. the measured MSD to the equation  $A(1 - \frac{1}{n})$  in order to obtain  $A$ , as was previously performed in Figure 2(b). The diffusion coefficient  $D$  could then be recovered by using  $D = \text{MSD}/(4 \times \text{time step})$ , noting that the diffusion can only be observed in two dimensions. It is still necessary to use the three-dimensional version of the Stokes-Einstein relationship to obtain the correct viscosity however.

The results can be seen in Table I. Since literature values for solutions at 286 K were not available, values were obtained by performing a linear interpolation using the values for 293 K, 303 K, and 313 K in Comesana *et al.*<sup>16</sup> Measured values are in broad agreement with the literature values, and notably more accurate than the measurements performed using just two particles, demonstrating the value of this approach.

In conclusion, provided the number of tracked particles is large, the error factor of  $(1 - \frac{1}{n})$  is negligible. In systems with only a small number of particles though, or where small groups of particles are processed separately (such as in systems that are known to be heterogeneous on large

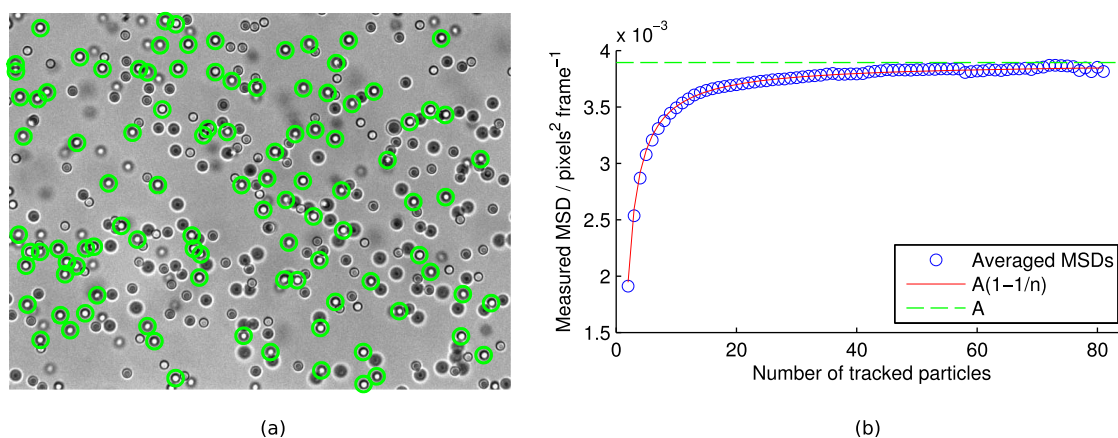


FIG. 2. (a) An example frame with circles highlighting the automatically identified and tracked particles. (b) Confirmation of theoretical predictions by experimental data. Because the MSD is dependent on both the particle and the liquid, the data were fitted to a curve given by  $A(1 - 1/n)$  where  $n$  is the number of tracked particles and  $A$  is the measured MSD with an infinite number of tracked particles. In this case,  $A = 0.003895$ .

TABLE I. Comparisons of measured and literature viscosity values.

Concentration of D-glucose in water	0 mM	2 mM	4 mM
Literature value <sup>a</sup> (mPa · s)	1.1	2.8	7.1
Measured value (mPa · s)	1.0	2.3	7.2
Measured value using only two particles (mPa · s)	2.0	5.8	15

<sup>a</sup>Literature values were interpolated from results in Comesana *et al.* (Ref. 16).

length-scales), this factor can become very large, and the mean-squared displacement can be underestimated by as much as 50%. Fortunately, correcting for this error is very simple, and consequently, it is hoped that the correction can be readily implemented in both existing and new particle tracking codes.

P. T. C. So acknowledges support from NIH 9P41EB015871-26A1, R01-EX017656, 5 R01 NS051320, 4R44EB012415-02, and NSF CBET-0939511, the Singapore-MIT Alliance 2, the BioSym IRG of Singapore-MIT Alliance Research and Technology Center, and the MIT SkolTech initiative. C.J.R. is grateful for a Wellcome Trust MIT Postdoctoral Research Fellowship to carry out

this research; this work was supported by the Wellcome Trust 093831/Z/10/Z. The authors are both grateful to Dr. Adam Corrigan for providing his MATLAB code and for his many fruitful discussions over email.

- <sup>1</sup>T. M. Squires and T. G. Mason, *Annu. Rev. Fluid Mech.* **42**, 413 (2010).
- <sup>2</sup>D. Weihs, T. G. Mason, and M. A. Teitell, *Biophys. J.* **91**, 4296 (2006).
- <sup>3</sup>T. A. Waigh, *Rep. Prog. Phys.* **68**, 685 (2005).
- <sup>4</sup>T. G. Mason, A. Dhople, and D. Wirtz, *Mater. Res. Soc. Symp. Proc.* **463**, 153 (1996).
- <sup>5</sup>J. C. Crocker, M. T. Valentine, E. R. Weeks, T. Gisler, P. D. Kaplan, A. G. Yodh, and D. A. Weitz, *Phys. Rev. Lett.* **85**, 888 (2000).
- <sup>6</sup>T. Savin and P. S. Doyle, *Phys. Rev. E* **76**, 021501 (2007).
- <sup>7</sup>A. J. Levine and T. C. Lubensky, *Phys. Rev. E* **63**, 041510 (2001).
- <sup>8</sup>T. Savin and P. S. Doyle, *Biophys. J.* **88**, 623 (2005).
- <sup>9</sup>M. T. Valentine, Z. E. Perlman, M. L. Gardel, J. H. Shin, P. Matsudaira, T. J. Mitchison, and D. A. Weitz, *Biophys. J.* **86**, 4004 (2004).
- <sup>10</sup>V. Pelletier, N. Gal, P. Fournier, and M. L. Kilfoil, *Phys. Rev. Lett.* **102**, 188303 (2009).
- <sup>11</sup>M. L. Gardel, F. Nakamura, J. H. Hartwig, J. C. Crocker, T. P. Stossel, and D. A. Weitz, *Proc. Natl. Acad. Sci.* **103**, 1762 (2006).
- <sup>12</sup>T. Moschakis, B. S. Murray, and E. Dickinson, *Langmuir* **22**, 4710 (2006).
- <sup>13</sup>J. Yang, C. Xu, P. Kopeckova, and J. Kopecek, *Macromol. Biosci.* **6**, 201 (2006).
- <sup>14</sup>A. M. Corrigan and A. M. Donald, *Soft Matter* **6**, 4105 (2010).
- <sup>15</sup>W. C. Navidi, *Statistics for Engineers and Scientists*, 2nd ed., edited by M. S. Hackett (McGraw Hill, New York, 2008).
- <sup>16</sup>J. F. Comesana, J. J. Otero, E. Garcia, and A. Correa, *J. Chem. Eng. Data* **48**, 362 (2003).