Electronic Supplementary Material (ESI) for Soft Matter.


# Effect of Photon Counting Shot Noise on Total Internal Reflection Microscopy

## Supplementary Information

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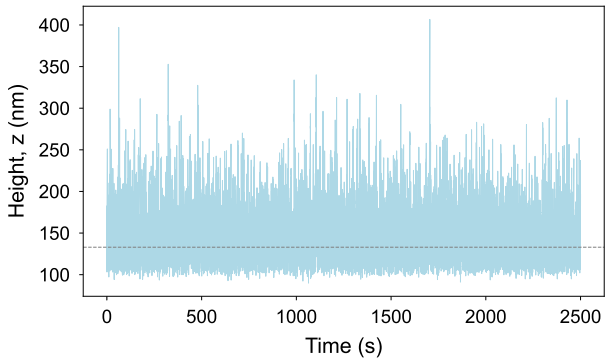


**Figure S1:** Simulated height trajectories of an 8.0-$\mu$m PS sphere in 0.5 mM NaCl. Grey horizontal line indicates the height corresponding to the potential minimum, 133 nm. Step size is 0.2 ms with $2.5\times10^6$ steps. Data are plotted from every 1000 points.

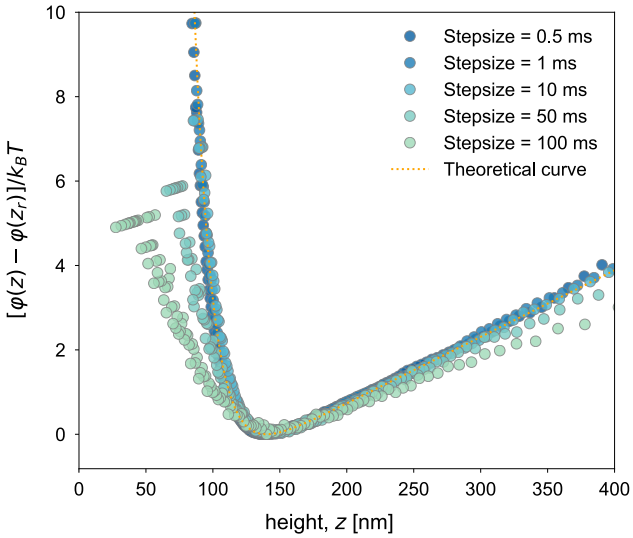


**Figure S2:** The effect of step size on the BD simulations. A temporal step size smaller than 10 ms is sufficiently small to simulate the movement of an 8.0-$\mu$m PS sphere in 0.5 mM NaCl.

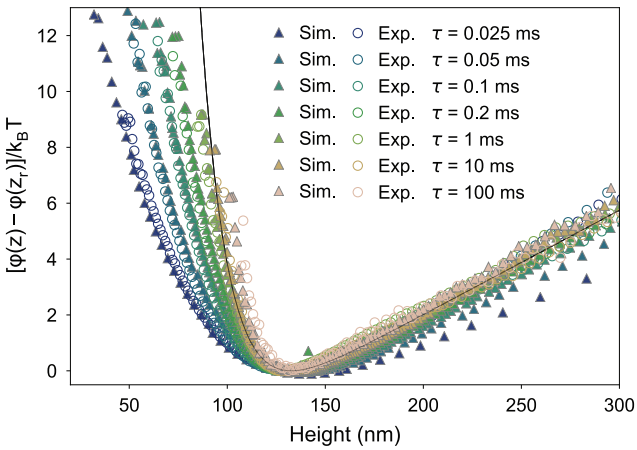


**Figure S3:** The simulated (triangles) and the experimentally measured (circles) potential curves of an 8.0-$\mu$m PS particle in 0.5 mM NaCl solution with different integration time. Dotted line shows potential $\varphi(z)$ used as an input for the simulation with $\kappa^{-1}$ = 13.7 nm and $G$ = 0.152 pN.

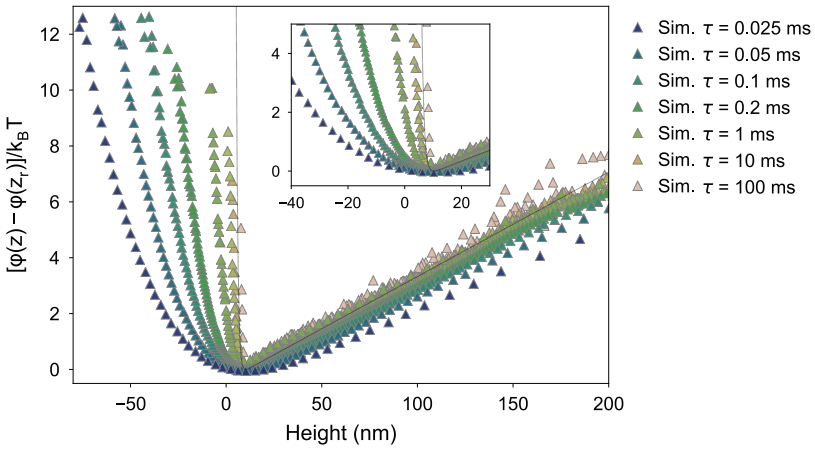


**Figure S4:** The simulated (triangles) and the experimentally measured (circles) potential curves of an 8.0-$\mu$m PS sphere in 140 mM NaCl solution with different integration time. Dotted line shows potential $\varphi(z)$ used as an input for the simulation with $\kappa^{-1}$ = 0.82 nm and $G$ = 0.152 pN. Inset shows the zoomed-in view of shot noise distortion at smaller separation distance.

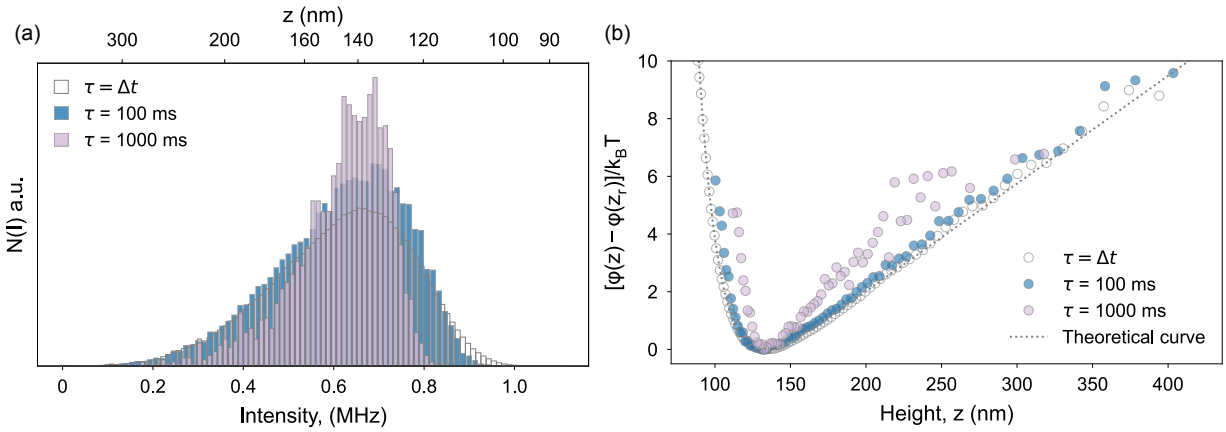


**Figure S5:** (a) Scattering intensity histograms from a simulation (step size, $\Delta$t is 0.2 ms with $2.5 \times 10^6$ steps) of an 8.0-$\mu$m PS sphere in 0.5 mM NaCl solution with photon counting interval of 100 ms (blue) and 1000 ms (purple). Grey bars are intensities from original simulated trajectories without account for shot noise or photon counting interval (effectively $\tau = \Delta$t). (b) Potential curves derived from histograms in (a) for $\tau$ = 0.2 ms (empty), 100 ms (blue) and 1000 ms (purple). Dotted line shows potential $\varphi(z)$ used as an input for the simulation with $\kappa^{-1}$ = 13.7 nm and $G$ = 0.152 pN.

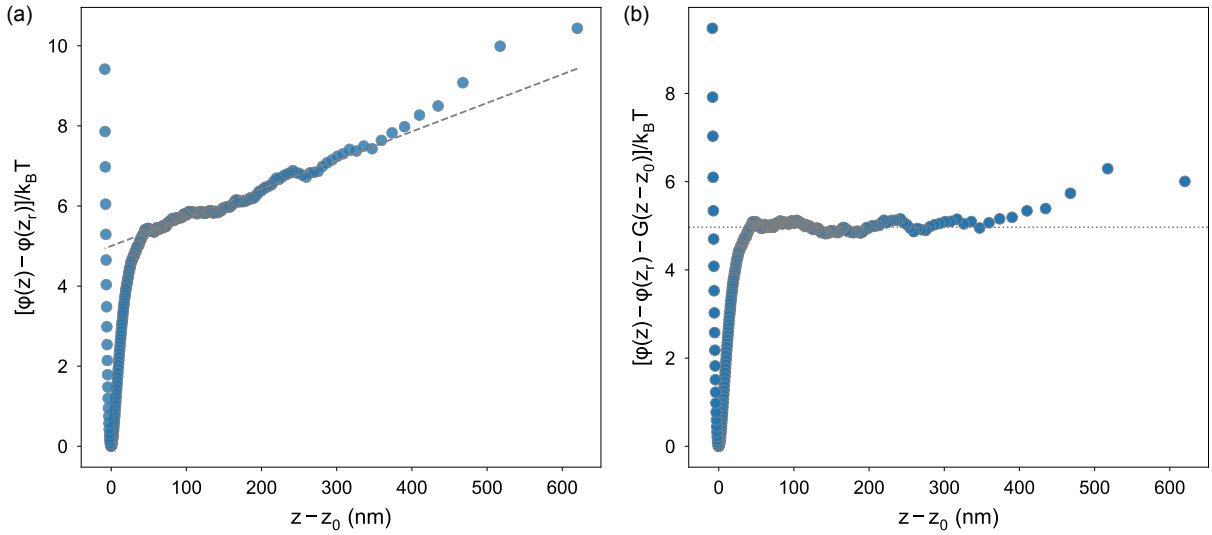


**Figure S6:** To assess the level of statistical noise caused a simulation of finite duration (2,500 s), shot noise is eliminated in the TIRM analysis of these data. (a) Simulated Morse potential for a 5-$\mu$m PS particle: $\epsilon = 5k_BT$, $a = 10$ nm, $z_0 = a$, step size $\Delta t = 5$ $\mu$s, $5 \times 10^8$ steps, $I_{max} = 1$ MHz. The integration time is set equal to the simulation step size: $\Delta t = \tau = 5$ $\mu$s. The dashed gray line shows the fit to the linear gravity term $G(z - z_0)$. The value of the buoyant weight $G$ is obtained by a linear fit to the potential curve at large separation distance ($z - z_0 > 100$ nm), with a weighting proportional to $\sqrt{N(I)}$, where $N(I)$ is the number of counts in the corresponding histogram of each data point. A value of $G = 0.0291$ pN is obtained by fitting the simulated data; a value of $G = 0.0371$ pN was used in the simulation. (b) The Morse potential with the gravity contribution removed. The depth is measured to be 4.97 $k_BT$ and shown with the dotted gray line. The full width half maximum (FWHM) is the potential well width at half of the extracted depth ($2.48k_BT$): 17.41 nm (the ideal value is 17.63 nm).

| $a = 10$ nm | Fitted $G$ (pN) | Fitted well depth ($k_BT$) | Fitted FWHM (nm) |
|---|---|---|---|
| # 1 | 0.0291 | 4.97 | 17.41 |
| # 2 | 0.0281 | 5.02 | 17.58 |
| # 3 | 0.0350 | 5.09 | 17.82 |
| # 4 | 0.0476 | 4.70 | 16.98 |

**Table S1:** Fitted parameters of Morse potential curves from separate simulation runs analyzed without shot noise. Simulations are performed for 5-$\mu$m PS particle with $\epsilon = 5k_BT$, $a = 10$ nm, $z_0 = a$. The step size is 5 $\mu$s with $5 \times 10^8$ steps. For the potential used in the simulation: $G = 0.0371$ pN, well depth is $5k_BT$, and FWHM is 17.63 nm. The data shown in Figure 6, 7 and Figure S6 are from simulation # 1.

| $a = 20$ nm | Fitted $G$ (pN) | Fitted well depth ($k_BT$) | Fitted FWHM (nm) |
|---|---|---|---|
| # 1 | 0.0391 | 5.25 | 36.86 |
| # 2 | 0.0449 | 4.51 | 32.62 |
| # 3 | 0.0434 | 4.61 | 33.22 |
| # 4 | 0.0242 | 5.38 | 36.35 |
| # 5 | 0.0229 | 5.69 | 39.03 |

**Table S2:** Fitted parameters of Morse potential curves from separate simulation runs analyzed without shot noise. Simulations are done for 5-$\mu$m PS particle with $\epsilon = 5k_BT$, $a = 20$ nm, $z_0 = a$. The step size is 5 $\mu$s with $5 \times 10^8$ steps. For the potential used in the simulation: $G = 0.0371$ pN, well depth is $5k_BT$, and FWHM is 35.26 nm. The data shown in Figure 7 are from simulation # 1.