Absolute Position Total Internal Reflection Microscopy with an Optical Tweezer - Supplementary Information

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I. SAMPLE CHAMBERS

For work with a high NA objective, and to damp out the effects of convection in the fluid for sensitive measurements of particle dynamics, in our work we use chambers with thicknesses on the order of 20 um.

As mentioned in the article text, the chambers are fabricated by pressing two strips of Solaronix sealing film between a glass slide and a glass cover slip and then heating to 120 C. Clamps are used to hold the glass slide and sealing strips in place during this process, and the amount of pressure, along with time in the oven, is an important factor in determining final chamber thickness.

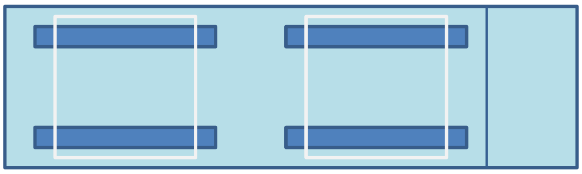


FIG. 1: Sample chambers are made two at a time and have thicknesses ranging from 15-20 um. Coverslips are glass with thickness number 1.5H. Samples are loaded by capillary action by dropping liquid onto either of the two open ends of each chamber.

II. DATA ACQUISITION

Scattered intensity data in the experiment is obtained in the form of photon counts per time interval. The time interval is set by the time between successive pulses of a pulse generator and changed according to the needs of the experiment. The APD operating in Geiger mode also sends a TTL pulse each time a photon is detected. The two signals go to a PCI-6602 counter by National Instruments, which sums the counts per designated time interval by using a common trick. APD ticks are used as a discrete "clock" to measure the length of the regular time-keeping pulses from the function generator. The output, the "period" of each time-keeping pulse, is simply the number of photon counts arriving during each set time-interval.

Its photon detection efficiency is about 50% at 550 nm and has a dead time of 77 ns after each pulse is sent. On the one hand, mean photon counts must be kept high enough so that the noise is not overwhelming, while on the other, the light source must not be too strong to avoid saturating the APD, resulting in lost counts.

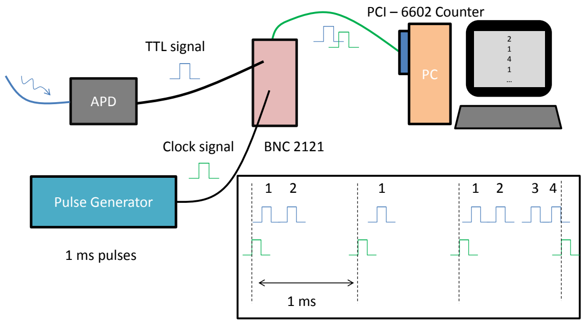
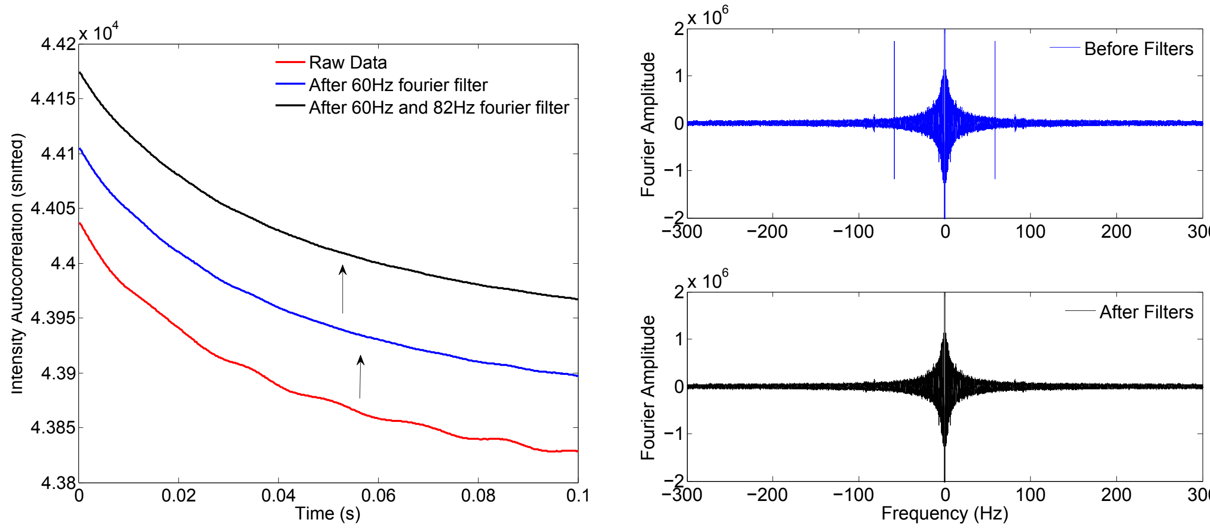


FIG. 2: In the data acquisition scheme, the PCI counter combines the signals from the pulse generator and APD to output data in the form of photons per time interval. For illustrative purposes a time interval of 1 ms is assumed.

III. FOURIER FILTERING

Since our lab is located on the first floor next to a busy road, our experimental environment is inherently noisy. After the usual precautions of upgrading and optimizing noise-isolating optical table legs, shutting off unit HVAC systems, and distancing vibration-generating components from our setup, we found it was still necessary on some occasions to Fourier filter our data for the extraction of the particle's diffusion coefficient.

We collect discrete APD count data, so we believe our noise to be mainly physical in origin, i.e., due to actual vibration of the bead or fluctuations in sensing or trapping laser intensity. We



(a) (b)

FIG. 3: a) Intensity autocorrelation function before and after narrow-band fourier filtering shown in steps. The curves are shifted vertically for clarity. b) Fourier spectrum before and after noise subtraction in the same two narrow bands.

found that within our data two main noise frequencies showed up quite consistently, the first at around 60 Hz and the second at around 82 Hz. 60 Hz likely corresponds to electronic noise, and 82 Hz was found to be due to a physical resonance of our microscope objective mount.

The closer the bead was brought to the glass surface, the more prominent these noise peaks became in the fourier spectrum of the data, presumably because the random noise was reduced along with the damping effect of the fluid. Because of this effect, the data at each piezo step was analyzed separately to confirm that the interfering noise peaks have been entirely removed.

Two figures are provided from the data analysis to illustrate our noise subtraction method. Figure 3(a) shows the intensity autocorrelation function before and after subtraction of the two above-mentioned noise bands, and Figure 3(b) compares the corresponding Fourier spectra.

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