Nanoparticle Diameter Measurements With Event Camera Tracking

Supplementary Material

8. Appendix

8.1. Modeling Brownian Motion

The process of Brownian motion can be modeled as a random walk [4, 7] where a particle's (2-dimensional) inter-frame displacements are taken from a zero-mean Gaussian distribution whose variance (σ^2) is related to the diffusion coefficient (D) by,

$$\sigma^2 = 4D\delta t^\alpha \tag{8}$$

where δt is the lag time (temporal spacing between centroid samples). In the case of solely diffusion-based mass transport, the exponent α is 1, and in anomalous diffusion (*e.g.*, confined spaces and situations with non-zero velocity fields), α is not equal to 1. The Stokes-Einstein relation [18] equates the diffusion coefficient of a particle to its hydrodynamic diameter,

$$D = \frac{k_B T}{3\pi\eta d} \tag{9}$$

where k_B is the Boltzmann constant, η is the dynamic viscosity and d is the hydrodynamic diameter. Combining Equations 8 and 9, the hydrodynamic diameter is expressed as a function of the physical parameters of the experiment and the variance of the displacements as

$$d = \frac{k_B T 4\delta t^\alpha}{3\pi\eta\sigma^2}. \tag{10}$$

Considering the central limit theorem [28], as the number of samples from the distribution of displacements increases the experimentally measured variance more closely approximates that of the underlying true variance. For a perfectly monodisperse sample, the measured CV is expected to nominally decrease with the inverse square root of track length, but the exact relationship depends on tracking precision [4, 26, 27]. Therefore, by increasing the sampling frequency of the particle motion, the measured diameter is expected to more closely approximate the true diameter. To test this hypothesis, we characterize a set of particles with known diameter and coefficient of variation (CV) to investigate the behavior of diameter measurement with larger ensembles of statistics. Equation (8) is commonly fit with a positive non-zero y-intercept (ϵ) that is proportional to the square root of the localization error to [26, 31] (see Figure 7):

$$\sigma^2 = 4D\delta t^\alpha + \epsilon. \tag{11}$$

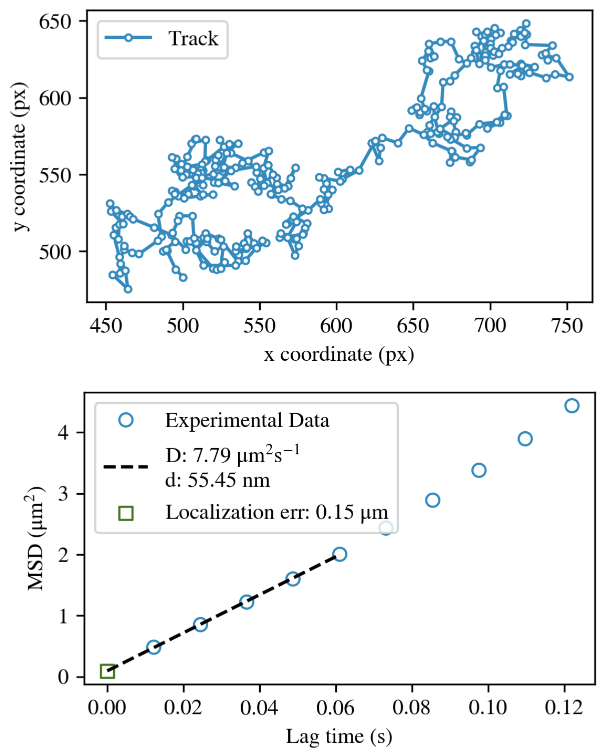

Figure 7. Data reduction from tracks to diameter. The top shows the tracks in the event camera coordinate system (0.06 μm px^{-1}) and the bottom shows the MSD curve with the linear fit for diffusion coefficient (D) and diameter (d). Data are from 80× magnification frame camera dataset.

8.2. 1D Event Filters

The kernels that were used in this work are shown in Figure 8 where each kernel is normalized with its discrete sum equal to 1 ($\sum_{i=1}^{n} x(i) = 1$). The selected kernels are not exhaustive, but meant to demonstrate the impact of temporal weighting schemes for particle tracking with the event camera. Note the kernels shown here span the full range of the equivalent exposure time of the frame-based camera.

8.3. Experimental Settings

Figure 9 shows the microscope with the cameras mounted and sample illuminated. In our experiments, we used the following components as our hardware, software, and samples. The hardware components are listed below (see Section 7).

- Event camera: SilkyEvCam HD; model: EvC4A; brand: CenturyArks; sensor: IMX636; pixel size: 4.86 μm; spa-

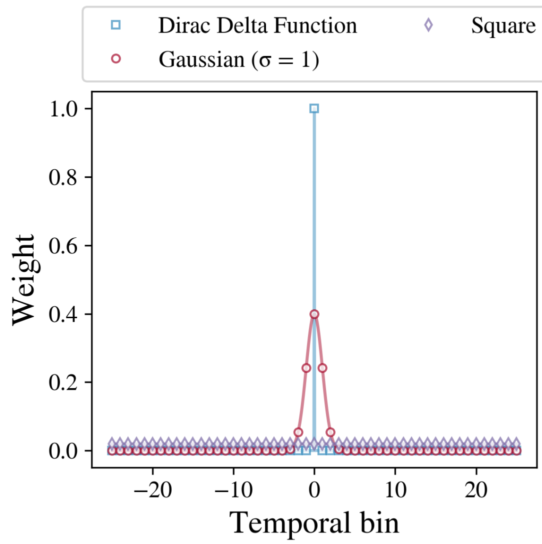

Figure 8. 1D temporal weighting kernels considered in this work

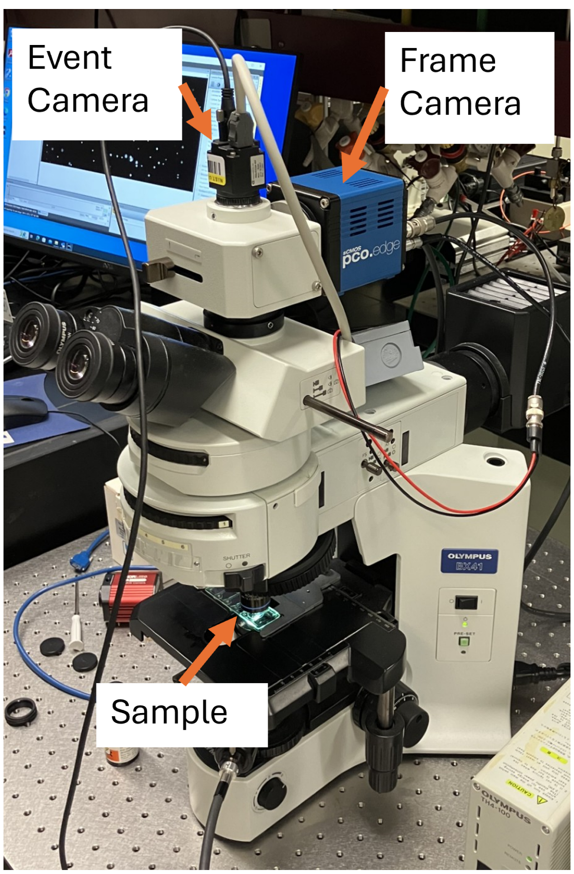

Figure 9. Microscope experimental setup.

tial resolution: 720 × 1280 pixels. The IMX636 sensor has a typical latency of less than 100 µs [6].

- Frame camera: model: PCO Edge 4.2 LT; pixel size: 6.5 µm; spatial resolution: 2048 × 2048 pixels; rolling shutter
- Microscope: Olympus BX-41 upright microscope with a 40×, 0.75 numerical aperture (NA) objective, 2x magnifier with an enhanced dark-field condenser (Cytoviva, Auburn, AL).
- Camera mounts: Olympus U-DPCAD double port adapter with silver sputtered non-polarizing 50:50 beam splitter dual port (see supplementary Figure 9).

The frame-based camera was operated at 82 frames per second (FPS) which constrained the field of view (FOV) from a height of 2048 pixels to a height of 1000 pixels to sustain the frame rate. The cameras were temporally synchronized with a trigger cable that conveyed the exposure and frame time intervals from the frame camera to the event camera.

8.4. Sample Images

Figure 10 shows some sample images from the datasets.

8.5. Field Of View Comparison

Figure 11 shows an overlay of frame and event camera fields of view (FOVs). The PCO edge user manual reports the relationship between sensor size and frame speed. Extrapolating the relationship between sensor height as a function of frame rate up to 4100 FPS would yield a maximum sensor height of about 50 px. The PCO edge may be incapable of operating stably at this frame rate (we did not test this), but we would expect a tradeoff in sensor size of this magnitude to achieve the equivalent tracking rate of the event camera. Although the FOV of the frame-based camera is larger at 82 FPS (Figure 11), the event camera would have a factor of 9 more pixels at 4100 FPS, where the frame camera would have a cropped sensor size of 2048 × 50 px.

8.6. Sampling at Increased FPS

For completeness Figure 12 and Figure 13 show the diameter convergence as a function of increasing track length at 60× and 40× magnifications.

8.7. Dataset Size

The hdf5 compressed datasets for the event camera were 846 MB, 853 MB, and 806 MB for the 40×, 60× and 80× datasets respectively compared to the 7.7 GB fixed dataset size of the frame-based camera. Normalized by the number of pixels this comes to 0.92 kB per pixel, 0.93 kB per pixel, 0.87 kB per pixel for the respective event camera datasets and 3.75 kB per pixel for the frame camera. The event camera represents x and y coordinates with unsigned 16-bit integer representation to span values up to 720 and 1280 respectively, while the polarity is Boolean and the time stamp is a 64-bit integer. The file size for the event camera is a

trackpy function: parameter	80× event	80× frame	60× event	60× frame	40× event	40× frame
batch: mpp (µm pixel^{-1})	0.061	0.061	0.081	0.081	0.122	0.122
batch: fps (tracking frequency) (Hz)	4100	82	4100	82	4100	82
batch: minmass	0.5	5000	0.5	5000	0.5	5000
batch: diameter (px)	31	31	23	23	15	15
batch: threshold ($\times 10^{-3}$)	1	1	1	1	1	1
link: search_range (px)	20	25	15	20	10	15
link: memory	150	3	150	3	150	3
filter_stubs: threshold	100	25	100	25	100	25

Table 3. Trackpy [2] settings each parameter corresponds to the locate and link functions. See trackpy API for further details.

Parameter	80×	60×	40×
scale (px)	10	10	5
update factor	0.5	0.5	0.5
interpolation method	dist	dist	dist

Table 4. Parameters for event interpolation noise filter [21].

function of the events that are detected by the sensor so its file size cannot be determined *a priori* and would likely differ for different microscope configurations. Likewise, other particle features such as light scatter or fluorescence intensity can be quantified from frame-based images that have effectively no resolution in the event space, which highlights the utility of event cameras in conjunction with frame-based cameras and not necessarily as a replacement.

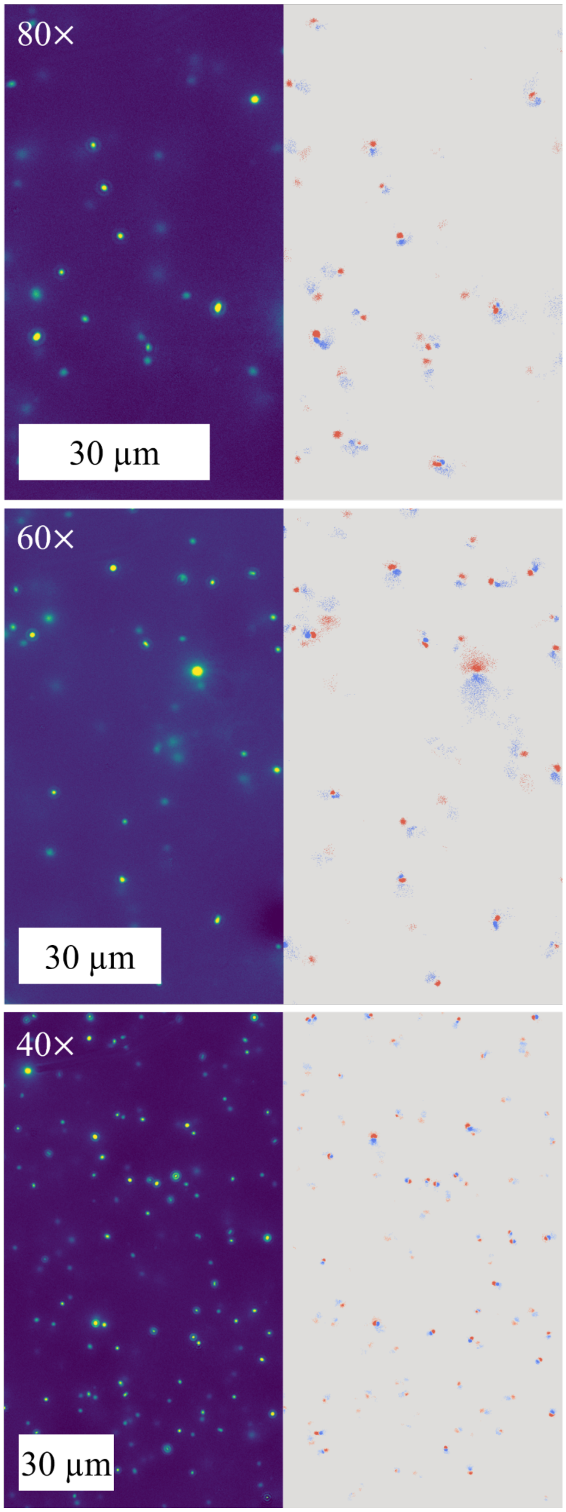

Figure 10. Spatio temporally registered camera signals (left: frame, right: event) at 80× (top), 60× (middle), and 40× (bottom).

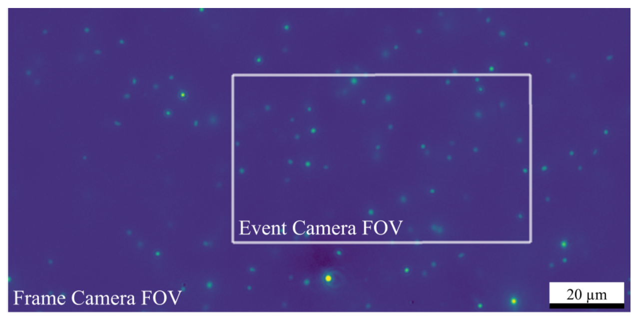

Figure 11. Comparison of camera FOVs.

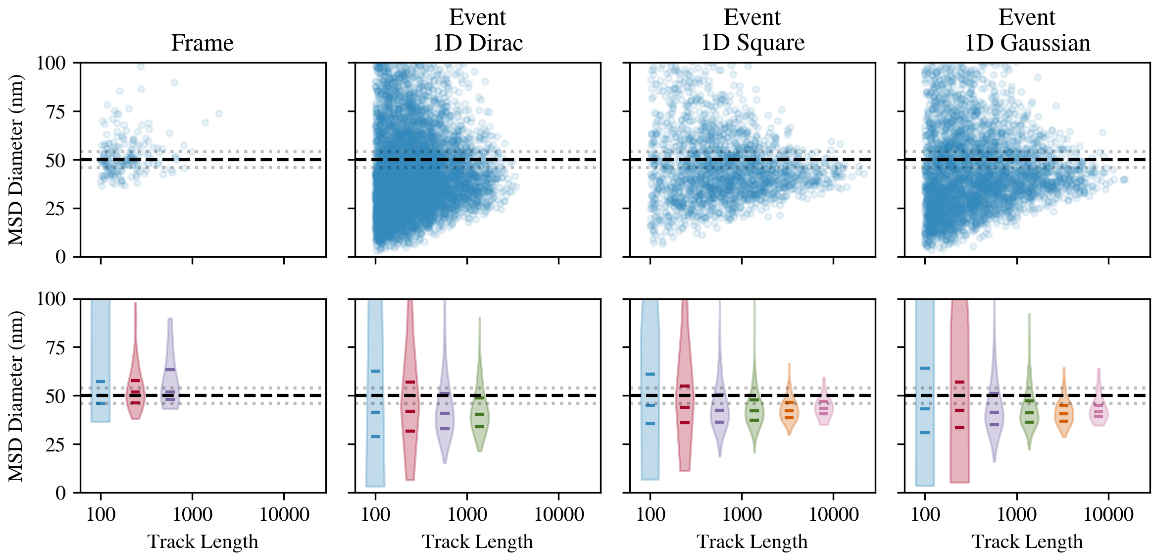

Figure 12. Comparison of tracking convergence between frame camera and event for 60× magnification.

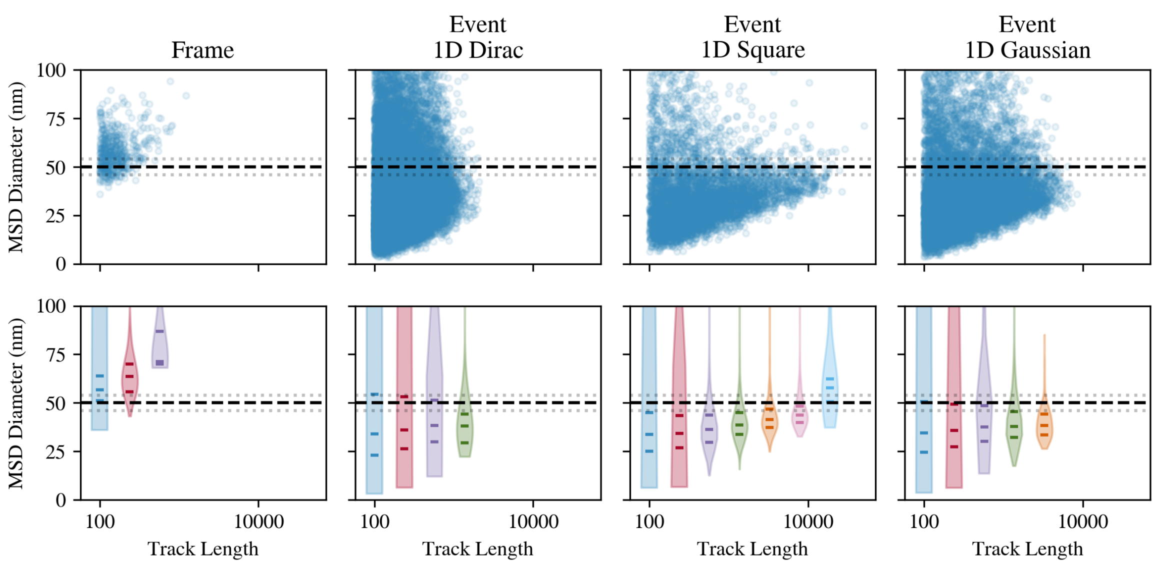

Figure 13. Comparison of tracking convergence between frame camera and event for 40× magnification.