Electronic Supplementary Information

Label-free, ultrahigh-speed, 3D observation of bidirectional and correlated intracellular cargo transport by coherent brightfield microscopy

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Email: clh@gate.sinica.edu.tw
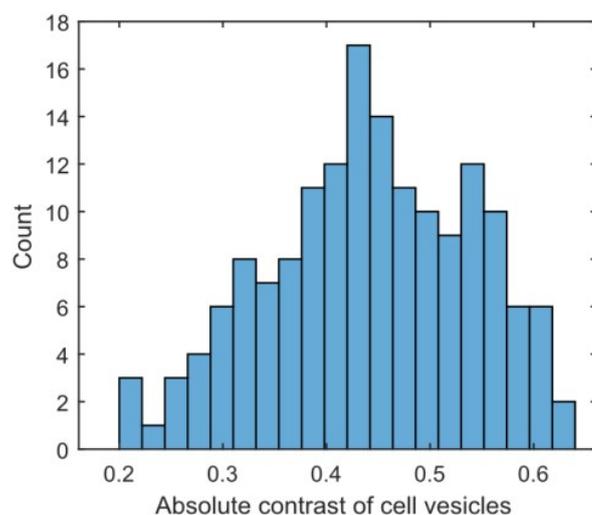
Fig. S1 Histogram of measured contrast of cell vesicles. Contrasts of 160 cell vesicles were measured, giving an averaged absolute contrast of 0.44 with a standard deviation of 0.10.
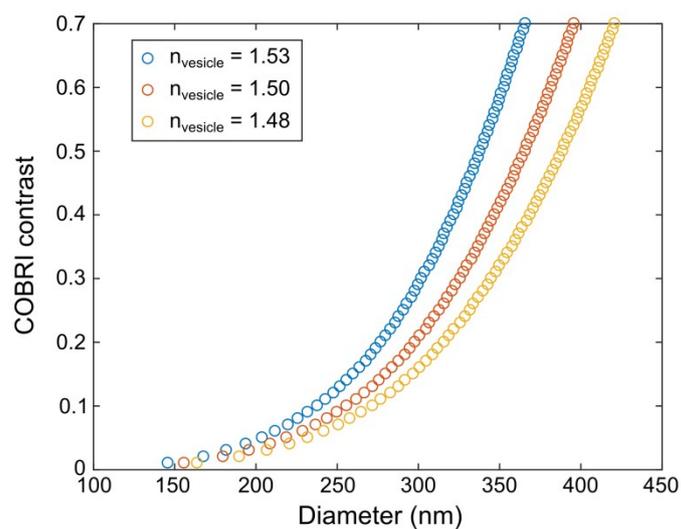
Fig. S2 Estimated dependency of the absolute optical contrast to the size of lipid-rich cell vesicle. We estimated the relationship between the optical COBRI contrast of the vesicle and its size by calculating the optical cross sections via Mie theory and the benchmarked measurements of gold nanoparticles of known sizes and cross sections (see Experimental Section for more details).
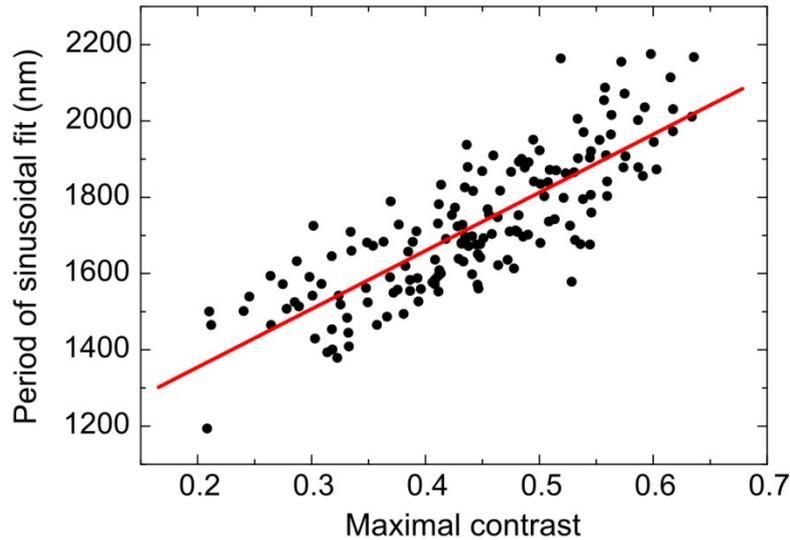
Fig. S3 Calibrations of height-sensitive CPBRI contrast of cell vesicles. The height-sensitive COBRI contrasts of 160 cell vesicles were individually characterized in fixed cell samples. The characterization procedure has been described previously.[1] Briefly, the axial position of the sample is modulated by a piezo stage and a series of COBRI images are recorded simultaneously. Each vesicle in the sample gives a sinusoidal calibration curve, from which the maximal contrast and a sinusoidal period are determined by fitting. This figure shows the relationship between the maximal contrast and the period of sinusoidal fit. There appears a positive correlation between the maximal contrast and the period, indicating that the change in particle size modifies the phase development of the forward scattering light. Here we phenomenologically describe the relationship between the maximal contrast and the period in the range of our interest by a linear fit (the red line in the figure). In the tracking experiment in live cells, we always measure the maximal contrast of the particle of interest by a quick z-scan of the particle. Given the maximal contrast of the particle, a sinusoidal calibration curve with corresponding period (defined by the linear fit) is used for reconstruction of the axial position of the particle. Discrepancy between the estimated calibration based on the linear fit and the exact calibration curve of the granule leads to distortion in the reconstruction of the axial position. We estimate the typical distortion is < 15 nm every 100-nm displacement.
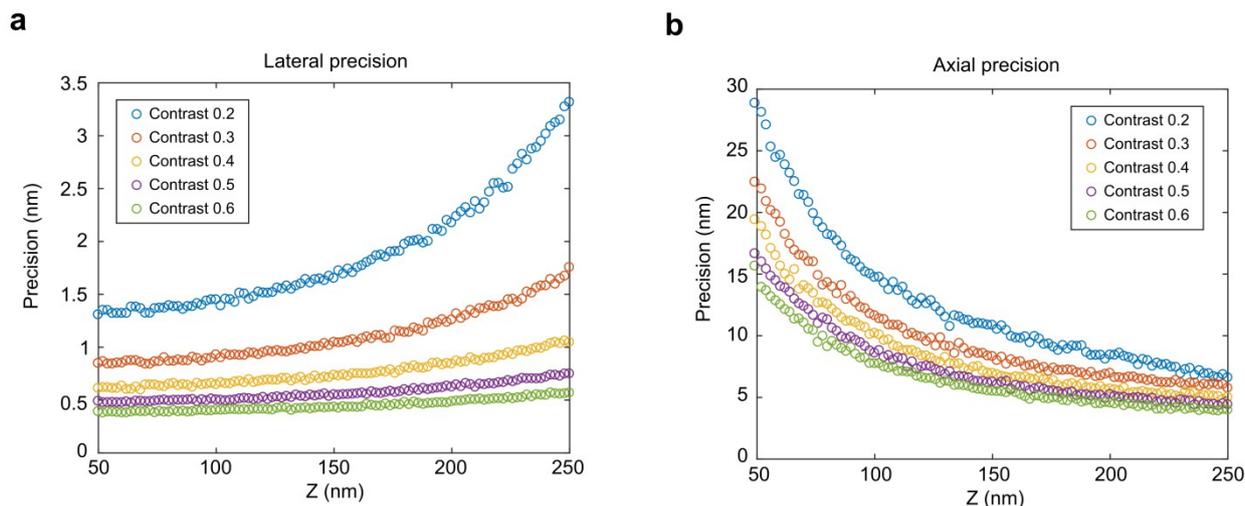
Fig. S4 Height-dependent localization precision. Lateral (a) and axial (b) localization precision as a function of particle height for different maximal particle contrasts. The precision was estimated by numerical simulations where the experimentally measured height-dependent contrast (as shown in Fig. S3) was considered. See Experimental Section for more details. The $Z = 0$ nm is defined as the height where the particle has the strongest contrast.
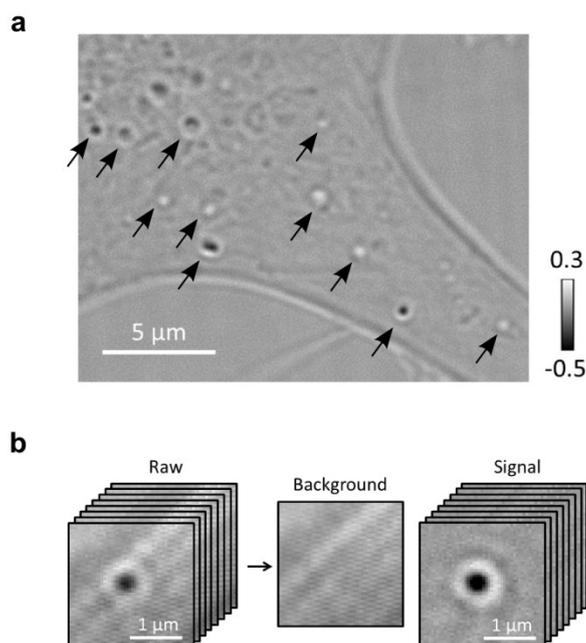
Fig. S5 Effect of background correction. (a) Raw COBRI image of a fibroblast cell in which the lipid-rich vesicles appear as dark or bright spots (depending on their heights, indicated by arrows). (b) A close-up view of a vesicle in the raw image (left), background (middle), and background-corrected image (right). The background image was estimated by temporal median filtering the image series where the background was relatively stationary compared to the diffusive and dynamic vesicle of interest.[1]
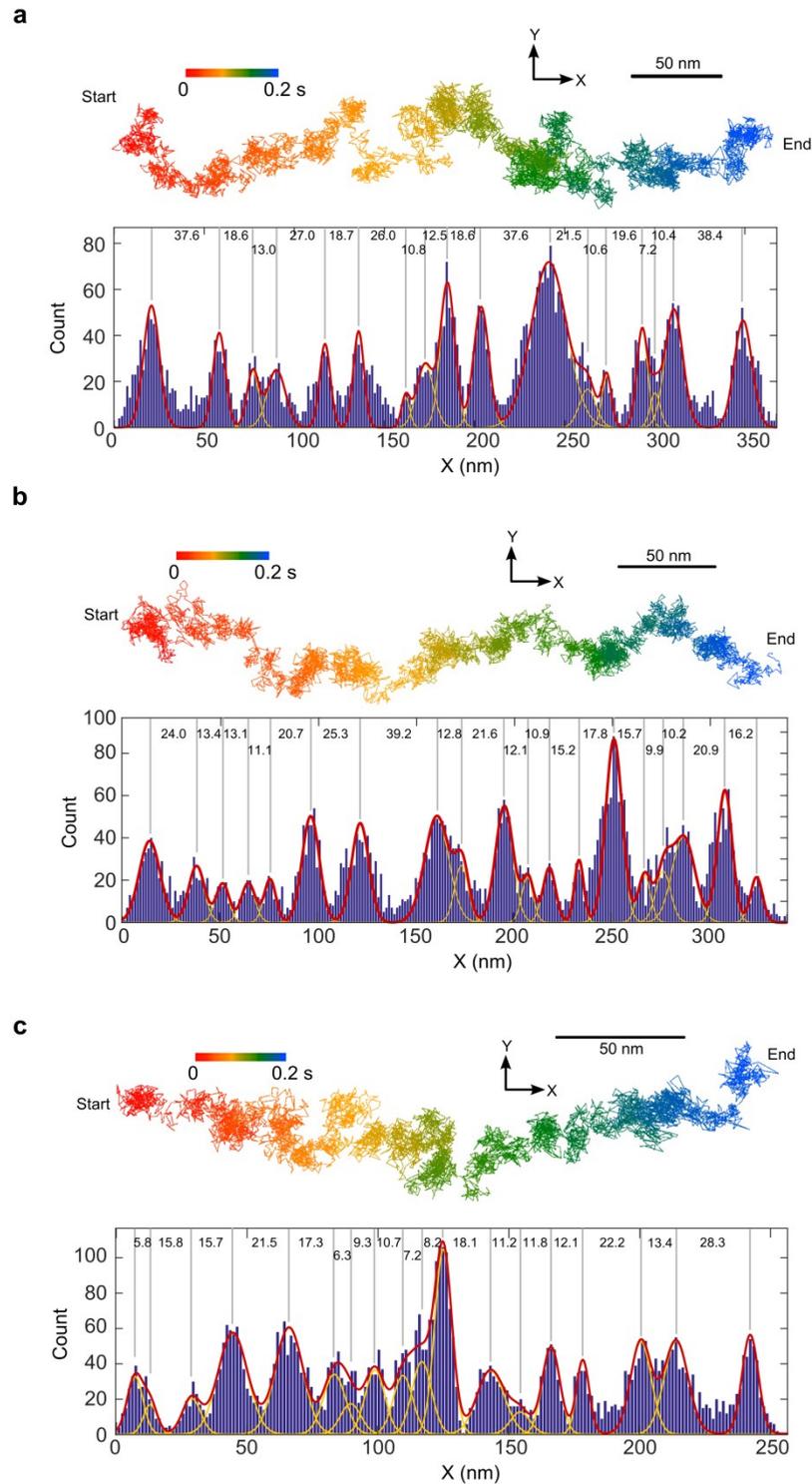
Fig. S6 Trajectories and analyses of stepwise motion of cell vesicles. (a)-(c) Three representative trajectories and histograms of their displacements showing clear stepwise motions. The data was acquired at 30,000 fps, giving 6,000 localizations in an observation period of 200 ms. Clusters of localizations in the trajectories and peaks in the histograms indicate the motions are stepwise. Positions of individual peaks were determined by step-finding method (Experimental Section) and distance between the peaks were labeled in the figure (unit: nanometers).
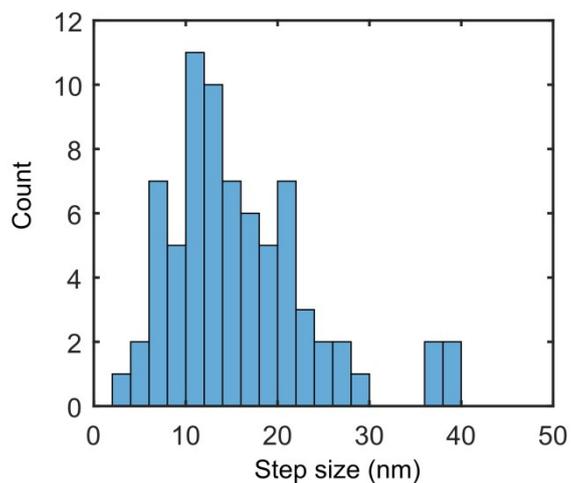
Fig. S7 Histogram of the measured step size. Based on the data of Fig. 2c and Fig. S6, the step size was found to be 15.9 ± 7.6 nm (mean ± standard deviation, N = 73).

Reference

1.   Huang, Y.-F.; Zhuo, G.-Y.; Chou, C.-Y.; Lin, C.-H.; Chang, W.; Hsieh, C.-L., Coherent Brightfield Microscopy Provides the Spatiotemporal Resolution to Study Early Stage Viral Infection in Live Cells. *ACS Nano* **2017**, 11, 2575-2585.