were 12-bit. Conversion from 12 to 8 bits was done in a way that retained the maximum number of graylevels in the moving particles. If this conversion had instead been done by simple division, the number of gray-scale increments defining the vesicle would have been severely reduced, adding digitization noise to the image noise.

MEDIC does not reduce shot noise or read noise present in the raw DIC image. If the images are digitized by a scientific-grade cooled CCD camera, shot noise will predominate. If the intensity is I at a particular pixel, the root-mean-squared (rms) shot noise is proportional to $\sqrt{I}$. The rms shot noise in the MEDIC background image is reduced to $\sqrt{I/8}$ if 8 frames are averaged. Thus rms shot noise in the background-subtracted image is $\sqrt{I+I/8}$, which is only 6% greater than $\sqrt{I}$. This is a small price to pay for the contrast enhancement provided by MEDIC (Table 1).

We have until now used MEDIC only with DIC microscopy. However, real-time background subtraction with rolling average generation of the background image may also be useful for enhancing the contrast of actively transported fluorescently labeled vesicles and organelles in live cells.

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Supplementary Materials includes 4 videos.

PC12_DIC.mov is a video of DIC images obtained with a live PC12 cell at 35-37C using a 60X water-immersion objective and cooled Hamamatsu ORCA ER camera with 6.4 x 6.4 μm^2 pixels, 8.3 fps frame rate, 12-bit digitization.

PC12_MEDIC.mov is a MEDIC video for a live PC12 cell. This video corresponds frame-by-frame to PC12_DIC.mov

Chick_DIC.mov is a video of DIC images obtained with a live chick motorneurons at 35-37C using a 60X water-immersion objective and cooled Hamamatsu ORCA ER camera with 6.4 x 6.4 μm^2 pixels, 8.3 fps frame rate, 12-bit digitization.

Chick_MEDIC.mov is a MEDIC video for a live chick motorneuron. It corresponds frame-by-frame to to Chick_DIC.mov.