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Title: Raw datasets of movies of single PolC-PAmCherry molecules in living *Bacillus subtilis* cells with high and low experimental background.

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Description: Single PolC-PAmCherry molecules were tracked in living *Bacillus subtilis* cells under high- and low-background experimental conditions. These movies can be used as a dataset to test the SMALL-LABS background subtraction algorithm.

Software:

The AVI movies can be played on most standard media players including Windows Media Player. The TIFF movies can be read by Matlab's *TIFFStack*. Both formats can be opened in ImageJ (a free commonly used image viewer and analysis program

Method: We used an IX71 inverted microscope (Olympus, Melville, NY) to image Bacillus subtilis strains on a 512 × 512 pixel Evolve electron-multiplying charge-coupled device detector camera (Photometrics, Princeton Instruments, Acton, MA). We excited with a 561-nm Sapphire 561-50 laser (Coherent, Bloomfield, CT) cells expressing the DNA polymerase PolC fused to the photoactivatable fluorescent protein PAmCherry as the sole source of PolC (strain JWS213). The low background condition contains only the desired fluorescence from the 561-nm imaging laser. We used a second, 488-nm laser to produce the high background condition: here a constant 15 W/cm^2, 488-nm laser (Coherent Sapphire 488-50) illumination generated a strong autofluorescent background in the cells; this background was further complicated by its slow decay over time. By stochastically switching a small subset (1 – 3 molecules per cell) of the PolC-PAmCherry molecules into a fluorescent state at a time (in a single-particle tracking/PALM experiment), we visualized the dynamics of single PolC-PAmCherry molecules in high-background cells and single PolC-PAmCherry molecules in low-background cells.

Date and location: These datasets were acquired in 2237 Chemistry in April 2017.

Files Contained Here:

low_bg.tif:
Uncompressed tiff stack of raw data from tracking single PolC-PAmCherry molecules in living Bacillus subtilis cells with limited background. Movies are acquired under continuous 561-nm laser excitation at a rate of 40 fps. Scale bar = 1 μm. This movie corresponds to Figure 3a in the main text.

low_bg.avi:
AVI version of low_bg.tif.

high_bg.tif:
Uncompressed tiff stack of raw data from tracking single PolC-PAmCherry molecules in living Bacillus subtilis cells with a high-background. A constant 15 W/cm^2, 488-nm laser illumination generated a strong autofluorescent background in the cells. Movies are acquired under continuous 561-nm laser excitation at a rate of 40 fps. Scale bar = 1 μm. This movie corresponds to Figure 3b in the main text.

high_bg.avi:
AVI version of low_bg.tif.

Related Publication: B.P. Isaacoff, Y. Li, S.A. Lee, and J.S. Biteen, "SMALL-LABS: An algorithm for measuring single molecule intensity and position in the presence of obscuring backgrounds," submitted to *Biophysical Journal* on April 18, 2018.