Materials and Methods

For details of Materials and Methods see (*1*). In terms of microscopy, here we used an Andor Model DV-860 BV, which is a back-illuminated 128 x 128 pixels with 24 uM pixel size, and binned it at 2 x 2 to achieve 1 msec per frame. The cells were kept at 10° C. Further detail are described in the figure legends of supplemental materials.

Supplementary Data

Fig S1. The polarity of microtubules in the process of S2 cells was examined by expressing EGFP-EB1 fusion proteins which decorate (+) ends of microtubules. Expression of EGFP-EB1 was induced by addition of 30 μM of $CuSO_4$ overnight, then cells were plated on ConA coverslips, and stimulated to grow processes by 5 μM of cytochalasin D. Images were acquired using a Nikon Eclipse U2000 microscope, 60X 1.4 N.A. objective, and 100W halogen lamp, and analyzed by METAMORPH software (*2*). The temporal movements of EGFP-EB1 along processes were examined and recorded as vertical displacements using "kymograph" function in the same software. A total 204 EGFP-EB1-labeled microtubule tips in 52 thin processes (< 1μm) were analyzed, which were 90 % toward outwards and 10 % toward cell body. A total of 100 EGFP-EB1-labeled microtubule tips in 31 thick (>1 μm or with lumps) processes were analyzed, which were 60 % toward outwards and 40 % toward cell body (data not shown). (**A**) EGFP-EB1 fluorescence distribution; (**B**) a corresponding phase contrast image (**C**) kymograph showing directional movement of GFP-EB1 over time. Scale bar, 5 μm.

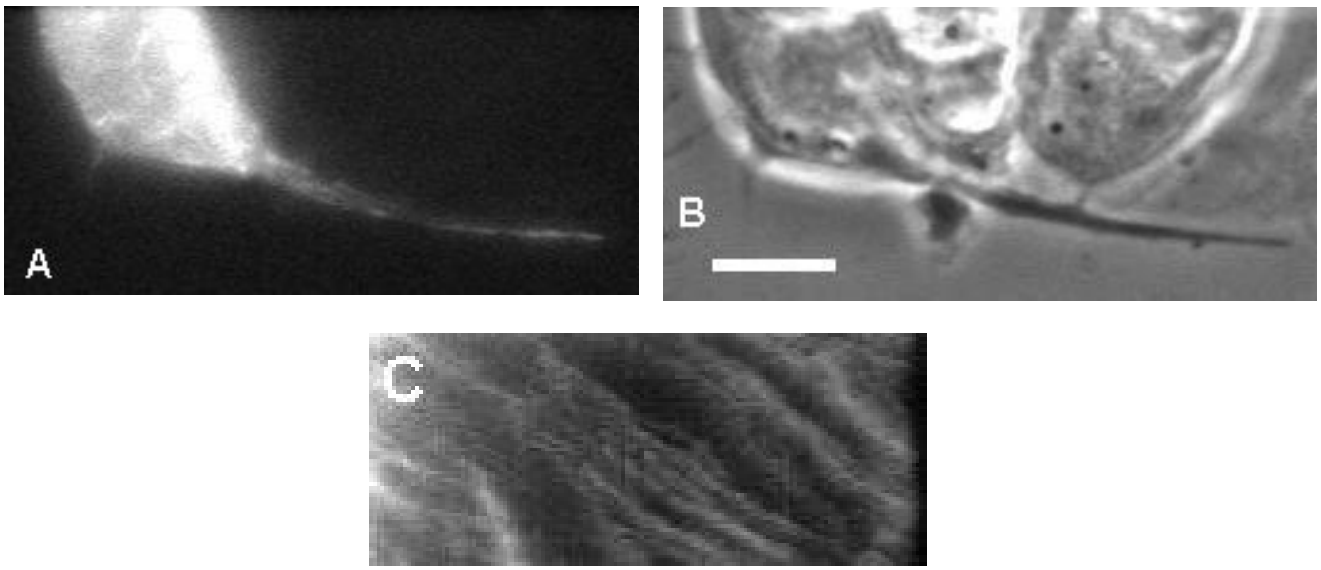
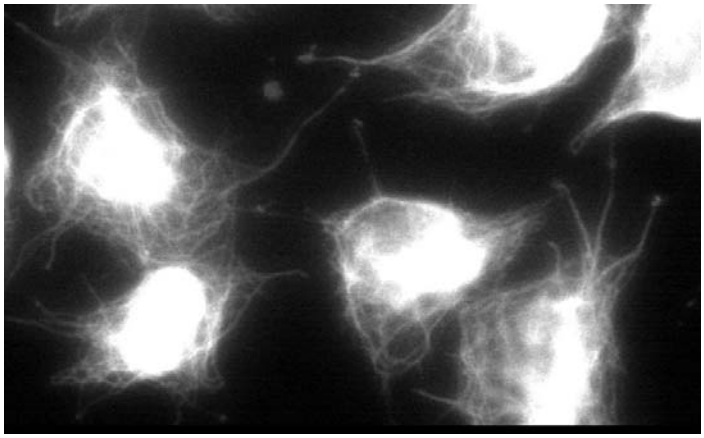


Fig. S2. Microtubules are stable at 10^{o}C. Microtubules in S2 cells are stable at 10^{o}C during recording. S2 cells were incubated at 10^{o}C for 1 hr, fixed with cold methanol. Cells are stained using alpha-tubulin monoclonal antibody, DM1alpha and Texas-red anti-mouse secondary antibody.



.

Fig S3. Effect of RNAi on GFP-peroxisome movements (**A**) Kinesin heavy chain/ dynein heavy chain (KHC/DHC) knockdown inhibit peroxisome transport about 10-fold compared to control. (**B**) Western blot analysis to confirm the specificity and efficiency of RNAi. The levels of KHC and DHC were reduced at least ten-fold after RNAi treatment. About 10^6 cells were incubated with 30 ug of dsRNA twice for 6 days and plated on ConA coverslips with 5 uM of cytochalsain D (*2*). Additional control RNAi assays for klp61F (Kinesin-5/Eg5 subfamily); klp53D, klp98A, and klp38B (Kinesin-3/Unc104 subfamily); klp68D, klp64D, and CG17461 (Kinesin-2 subfamily); ncd (Kinesin-14/C-terminal kinesin subfamily) were performed showing that there were no significant effects on the motility of peroxsisomes (data not shown, 13). Distribution of microtubule was intact after RNAi and actin was destroyed by cytochalasin D (data not shown).

(A)

RNAi Treatment	No. of moving particles	Total no. of particles scored	Relative % of movements	No. of cells analyzed	No. of peroxisomes tracked
Control	55	5010	1.1	8	83
KHC	0	3691	0	8	62
DHC	6	5311	0.11	11	89

The total number of particles scored is the number of peroxisomes seen on all frames during recording. The number of moving particles is the number of particles which moved with at least 0.1 um/sec. The relative % of movements is the ratio of number of moving particles to the total number of particles scored.

(B) Western:

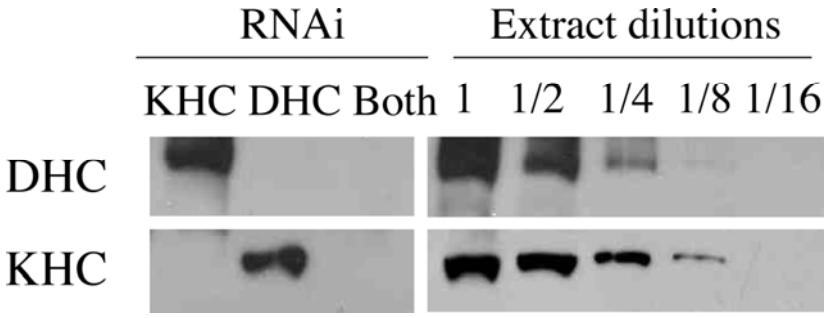
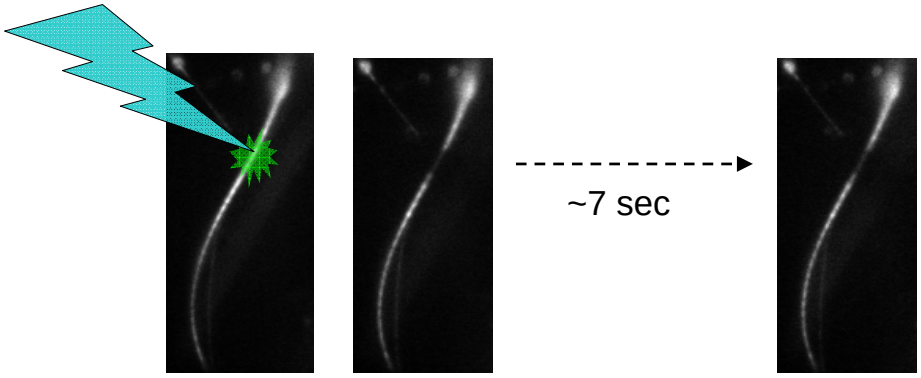


Fig S4. Photobleaching experiments made on S2 processes containing microtubules composed of EGFP labeled tubulins: No significant fluorescence leakage into photobleached area is observed 7 seconds after photobleaching for a second, showing that microtubular lattice within the processes are not moving with a significant speed. However, at 3 minutes after photobleaching, more than 70% fluorescence recovery is observed (Data not shown).



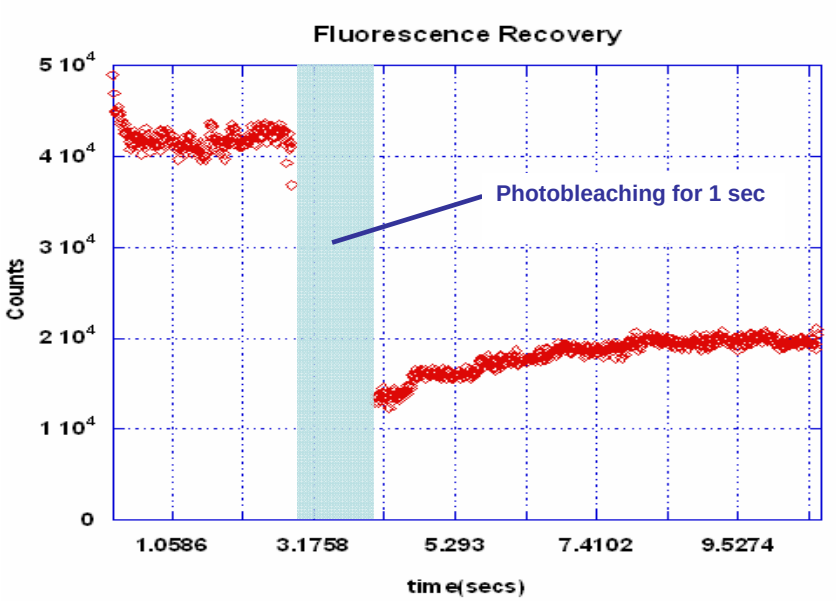


Fig S5. Effect of compliance on step-size in a tug-of-war between dynein and kinesin. With a compliance of 1 pN/nm for both dynein and kinesin, a single step by kinesin (16 nm per head; 8 nm per center-of-mass), moves the cargo (here containing GFP-peroxisome), only 4 nm.

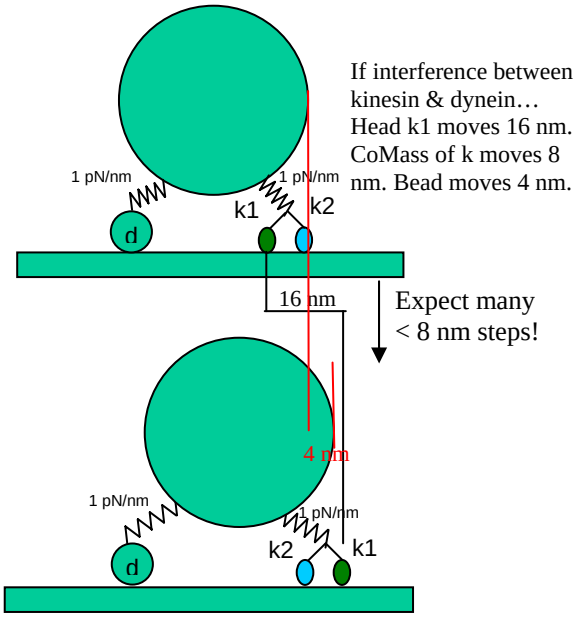
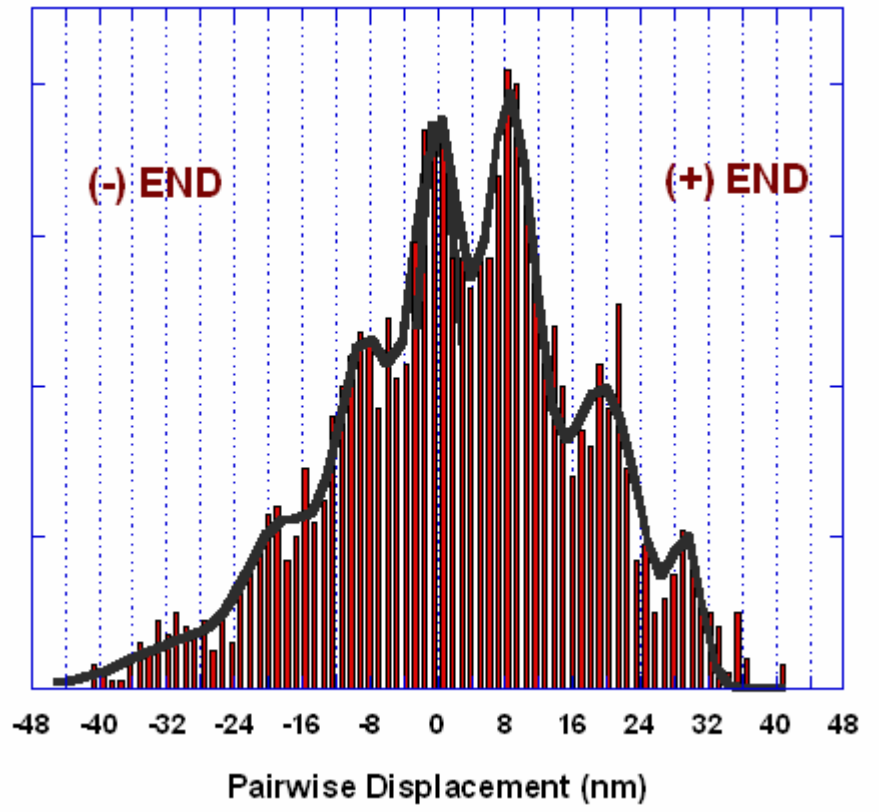


Fig S6. Pairwise displacement of kinesin and dynein, showing 8 nm steps for both.



References

1. A. Yildiz *et al.*, *Science* **300**, 2061 (2003).

2. S. C. Ling, P. S. Fahrner, W. T. Greenough, V. I. Gelfand, *Proc Natl Acad Sci U S A* **101**, 17428 (2004).