the expense of percentage time in catastrophe (**Fig. 1e**), apparent at the 3.3 nM concentration and above. At 1.0 Hz, percentage time in growth increases with Taxol concentration at the expense of percentage time in catastrophe but not pause (**Fig. 1e**), becoming statistically significant at the 10.0 nM level. The same treatment (indeed, the same data) acquired at different rates leads to opposing conclusions owing to artifacts introduced at slow acquisition rates.

Alternative microtubule tracking methods such as laminar whole-microtubule tracking show results agreeing with those from 4.0-Hz data[3,4]. These techniques capture the microtubule end position during all phases but are limited to isolated plus-ends near the cell periphery[5,6]. To avoid the introduction of artifacts in plus-end fluorescent-comet tracking, acquisitions of image stacks for microtubule plus-end dynamics analysis should be performed at the highest frame rate possible.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper (doi:10.1038/nmeth.2846).

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Danuser replies: Nicovich and Zhou[1] remind researchers in the cytoskeleton field of a well-known but often forgotten issue: parameters describing growth and shrinkage dynamics of cytoskeletal polymers—here, microtubules—vary with the frame rate of the image sequence. Given a time interval Δt between frames, any growth or shrinkage event lasting less than $2 \times \Delta t$ will be detected ambiguously. Whether it is still detected as a growth or shrinkage event depends on the actual duration and rate of polymer assembly or disassembly. Regardless, temporal undersampling causes underestimated rates of microtubule growth and shrinkage and underestimated rates of catastrophe (switch from growth to shrinkage) and rescue (switch from shrinkage to growth). Although the undersampling usually does not preclude detection of changes in microtubule dynamics between molecular backgrounds as long as imaging conditions are identical, measurements originating from image sequences sampled at, for instance, 5 seconds per frame cannot be compared to measurements from image sequences sampled at 1 second per frame.

To make their case, Nicovich and Zhou use the plusTipTracker software[2], which measures microtubule dynamics by tracking fluorescent particles that mark the selective association of fluorescent protein fusions to plus-end tracking proteins (+TIPs) at growing microtubule ends. The authors conclude that accurate particle tracking requires subsecond sampling. Clearly, the shorter the Δt, the lower the ambiguity in particle assignment between frames, and thus the less error-prone the tracking. However, with fluorescence live-cell imaging, this rule has a cautionary footnote: faster sampling accelerates bleaching, which is in addition to the more general concern that cell physiology may be disrupted. Thus, with too fast sampling, it may happen that +TIP markers are lost before a growth period has terminated. The good news is that growth speeds are largely unaffected; however, catastrophe rates may be substantially overestimated. Moreover, the plusTipTracker software offers the feature of interpolating pause and shrinkage events where the marker particle disappears. Premature bleaching leads to a considerable underestimation of the shrinkage speed and rescue frequency.

To alleviate this problem, computer scientists have put much thought into developing accurate tracking solutions despite an increased ambiguity when using lower sampling. plusTipTracker, especially the upgraded version[3], is equipped with a global optimization module for particle assignment and Kalman filter approaches that predict the immediate future of a microtubule plus-end trajectory on the basis of its past movement. This permits less frequent sampling; by how much depends on the image quality, microtubule density and microtubule dynamics. Defining the sweet spot between too fast and too slow sampling is a formidable task when starting a particle-tracking project. In Matov *et al.* we provide some practical guidelines (supplementary information in ref. 2). It should be noted that tracking parameters must be adjusted to the temporal sampling. Inspection of the data presented by Nicovich and Zhou (Fig. 1d in ref. 1) suggests that this may not have been fully accomplished. But this should not distract from the key message of this figure: parameters of microtubule dynamics cannot be interpreted without consideration of the sampling rate.

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Protein digestion priority is independent of protein abundances

To the Editor: Depletion of high-abundance proteins is an effective way to improve sensitivity in the identification of low-abundance proteins in shotgun proteomics. A recent Brief Communication by Fonslow *et al.* reported an interesting approach for improving proteome coverage by a method termed digestion and depletion of abundant proteins (DigDeAPr)[1]. Fonslow *et al.*[1] claimed that their method exploited the abundance-dependent Michaelis-Menten kinetics of trypsin digestion to selectively digest and deplete high-abundance proteins in