Supporting Info

Image Acquisition and Tracking

To detect the edges of the network, we developed a network tracking algorithm in Matlab that is broadly based on the idea of predictor-corrector algorithm.[32] As the network spreads out the signal to noise ratio decreases at the edges. This makes it difficult to robustly identify the edges from its surrounding over the duration of image acquisition. We overcome this problem by using an iterative tracking algorithm that approximately detects the edges of network and then successively improves its previous answer to converge to correctly identified edges. The first step of the algorithm uses an edge detection function to find the approximate edges of the network. We deliberately adjusted the threshold parameter of the edge detection function so that the detected edge lies completely inside the network. We visually verified that our choice of parameter was detecting only the interior of the network. The region that was detected as interior in the first step was masked out by setting it to zero. This new image is used as an input for the next round of edge detection and subsequent correction. This process is repeated several times until we are left only with true edges of the network. By tuning the threshold for edge detection and the number of iterations, we can detect edges robustly for all time points in the same time lapse video. As an example we show boundaries of a network tracked by our algorithm in Fig. S2

Longer Time Dynamics

Our classification of states as extensile, contractile, or static was based on observing dynamics of network for first 90 minutes. The longer time behavior of states can be different from our classification due to various reasons. First, at the time scale of a few hours the ATP in system runs out and the activity ceases. This affects the network regardless of its initial dynamic state. Second, a contractile network can go to static state after it contracts and increases its overlap enough to stall all the motion (as described in Fig. 4). This can depend on initial size of the cluster and the rate of contraction. In order to compare how the network dynamics changes over time, we compared the type of motion (extensile, contractile, or static) for two different time points at 15 min and at 45 min (Fig. S3). We found that only two positions in the state diagram changed from contractile to static.

Simulation details

We initialize the simulation by arranging microtubule filaments at a fixed distance from each other. As the simulation is set in one dimension, all the microtubules are positioned along the x axis and are oriented randomly with their direction of transport points either left or right with equal probability. For simplicity we choose all microtubule filaments to have same length 'l' and a fixed free speed V_{free}. At the beginning of each simulation step we calculate the local density ρ around a microtubule. For calculations of ρ, all neighboring microtubules are considered irrespective of their orientation. We also calculate the overlap O of a filament with its neighbors, but here we only use the filaments which are aligned antiparallel to the test filament. We then update the speed of the test microtubule according to equation 3 (in the main text). The position of the microtubule is then updated accordingly by moving it in its transport direction. The simulation is allowed to evolve for a total time which is an order of magnitude larger than the time that it takes to traverse the system size. We varied the crosslinker fraction 'f' from 0 to 0.5 in steps of 0.05. We varied line density of microtubule filament ρ_{MT} from 1 to 3 in steps of 0.25. The line density is defined as number of filaments per unit length of filaments.

Extensile states are trivial to identify. To distinguish between a contractile and a static state we used the following algorithm. We measured the number of microtubules remaining inside the initial starting volume and the mean distance traveled by them at the end of simulation. If more than 50% of microtubules are inside the initial starting volume and have traveled a mean distance significantly larger than the body length of a microtubule, then we classify this state as contractile.

If the mean distance traveled by microtubule is smaller than its body length, then we classify the states as contractile. The statistics of simulation are shown in fig. 5. The parameters used to simulate equation 3 (from the main text) are given in table 1

Table 1: Table caption

Parameter	**Value**
Length of filament (L_{MT})	20.0 μm
Free speed of filament (V_{free})	10.0 μm/s
Number of filament (N)	100
Maximum overlap allowed (O_{max})	100 μm
Maximum local density (ρ_{max})	1 μm^{-1}
Time step	0.1 s
Number of time steps	1000
Number of iterations	100

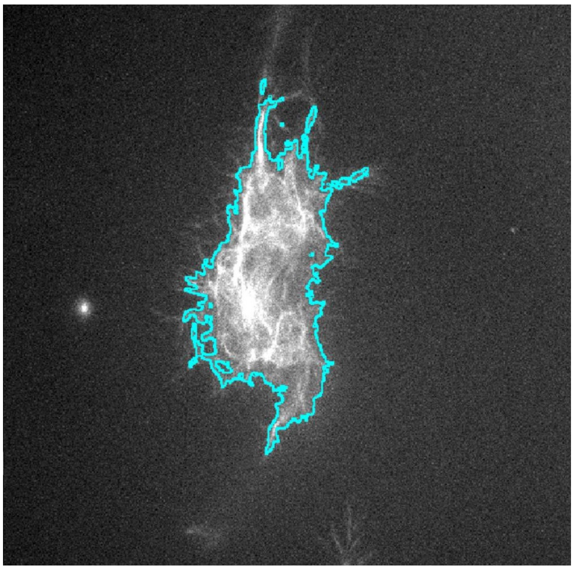
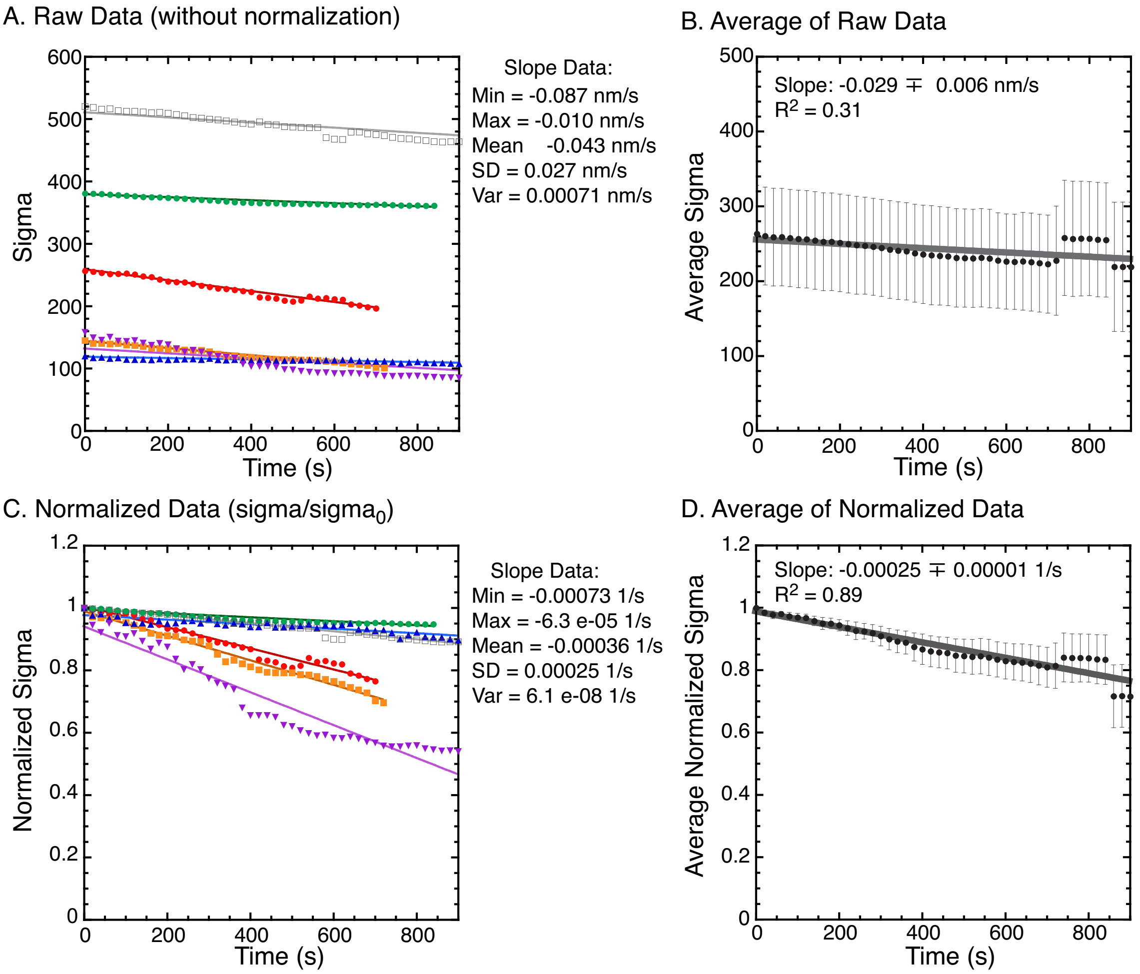


Figure 1: Example network with the edges detected. The detected points on the edges are colored in Cyan. The locations form the set x_i used to calculate σ from equation 1 from main text. The quantity μ is spatial mean of points detected on edge. The variance, σ, is with respect to the mean, equation 1 in the main text.

Microtubule Concentration: 5 μM
MAP65 Percent Bound: 24%
Contractile Motion



A. Raw Data (without normalization)

Slope Data:
Min = -0.087 nm/s
Max = -0.010 nm/s
Mean -0.043 nm/s
SD = 0.027 nm/s
Var = 0.00071 nm/s

B. Average of Raw Data

Slope: -0.029 ∓ 0.006 nm/s
R^2 = 0.31

C. Normalized Data (sigma/sigma$_0$)

Slope Data:
Min = -0.00073 1/s
Max = -6.3 e-05 1/s
Mean = -0.00036 1/s
SD = 0.00025 1/s
Var = 6.1 e-08 1/s

D. Average of Normalized Data

Slope: -0.00025 ∓ 0.00001 1/s
R^2 = 0.89

Figure 2: Example data analysis for data with 5 μM microtubule concentration and 24% MAP65 binding. (A) The shape variance as a function of time, $\sigma(t)$ from six different networks. The slopes give the rate of change of the shape, v. The data reported gives the minimum, maximum, mean, standard deviation (SD) and variance (var) of the six data sets when fit to a line: $\sigma(t) = \sigma_0 + vt$. (B) When the data from part (A) are averaged and fit to a line, the standard error of the mean is very large for the averages, implying that there is no significant change for the data. The slope of the line is given. This large standard error is an effect of the data having all different initial starting sizes. The spread in the initial data masks the signal significance and adversely affects the goodness of fit. (C) When the data in part (A) is normalized by $sigma_0$, the data does not necessarily collapse, but the variance is far smaller than the raw data. (D) When the data from part (C) is averaged, the data is fit to a line, and the slope is given as a rate and the goodness of fit is given. For all the data

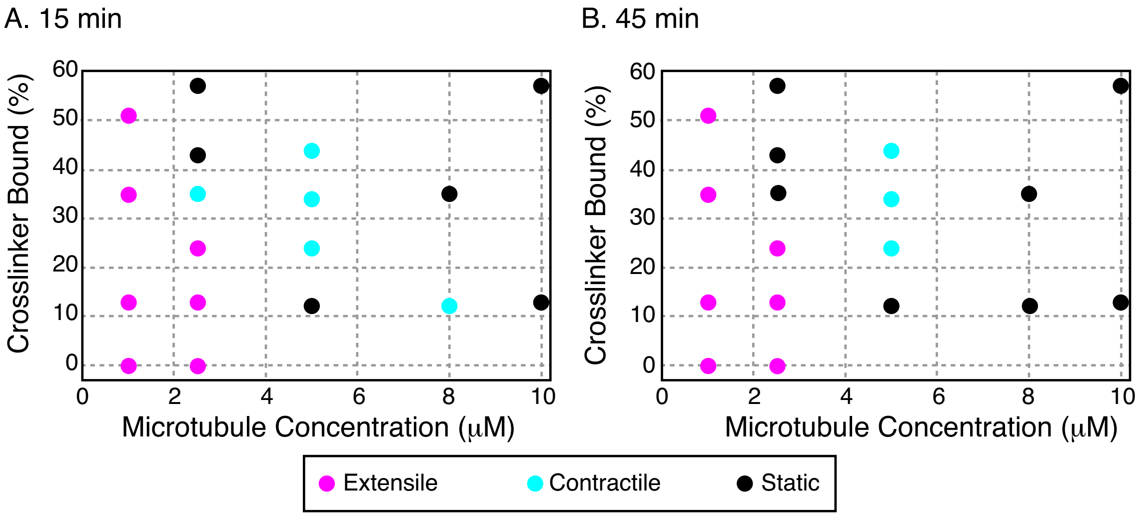


Figure 3: The dynamics of the network of bundles at two different time points. (A) Network dynamics type at 15 min into the time lapse imaging. (B) Network dynamics type at 45 min into the time lapse imaging. For both, network dynamics that are extensile are colored magenta, contractile networks are cyan, and static networks are black. Two of the contractile systems from 15 minutes became static at 45 minutes.

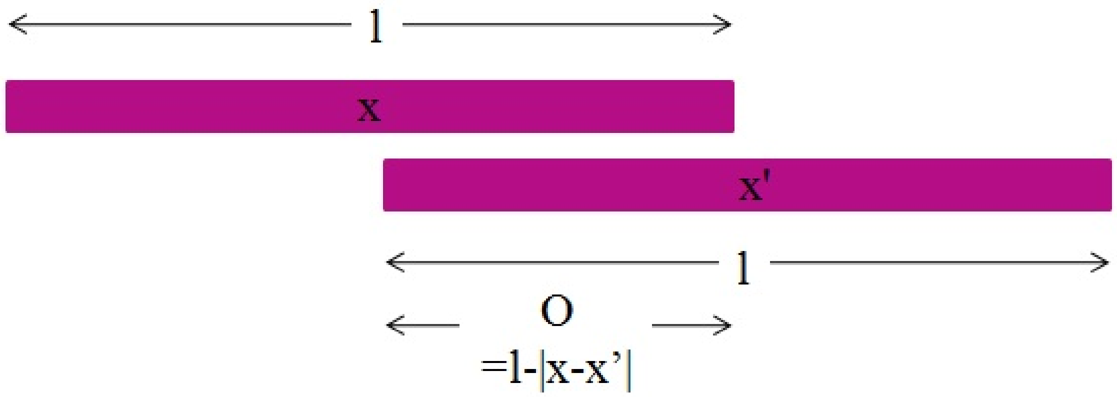


Figure 4: A schematic of the situation used in the simulations. The overlap, O, between two microtubules of length, l, with their centers at x and x'. The amount of overlap controls the velocity depending on the fraction of crosslinking proteins, f as in equation 3 of the main text.

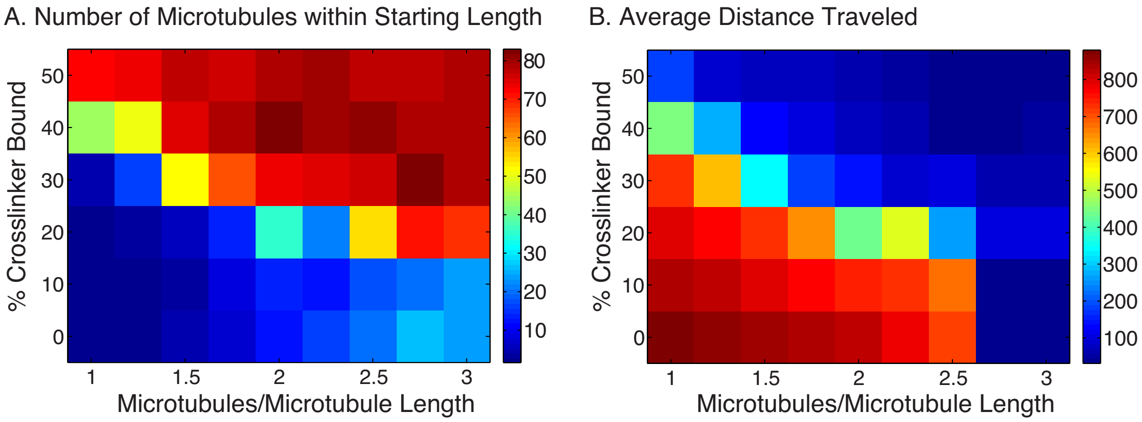


Figure 5: Simulation statistics used to classify dynamics of a cluster. (A) The number of microtubules within the initial starting length of the filament. (B) The average distance traveled by microtubules before coming to rest. The classification of the activity as extensile, contractile, and static depended on both of these parameters. Extensile networks have a low number of filaments in the same region because they all glided away. Extensile systems also have filaments with a high distance traveled because they all traveled out of the region. Static networks have a high density of microtubules that stay within the same region over time and travel the least. Contractile systems have an intermediate number of microtubules within the original region and also move an intermediate distance.