



Title	Monopolar flocking of microtubules in collective motion
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## **Supplementary Information for Monopolar flocking of microtubules in collective motion**

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This supplementary information includes,

Data analysis

Supplementary figures

Supplementary movie legends

## Data analysis

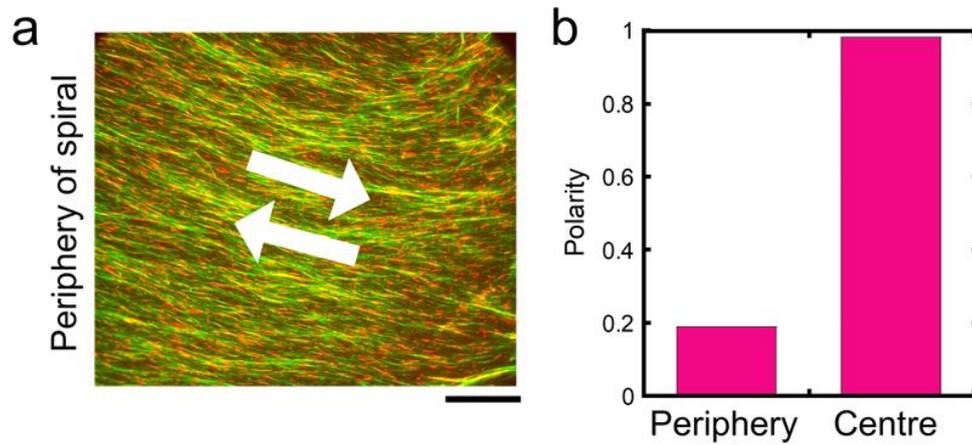
### Quantitative analysis of rotational angle of microtubules in collective motion

The fluorescence microscopy images were analyzed by NIS- Elements BR software (Nikon) and Fiji 1.52J software (National Institutes of Health, USA). Trajectory of microtubules and the moving direction or rotational angle  $\theta$  was measured for 50 microtubules at each 5-second video frame, using the ImageJ plugin ‘MTrackJ’ (<https://imagej.net/MTrackJ>). Left / right asymmetry of the moving direction of microtubules was quantified by the time average of  $\text{Cos}(90^\circ - \theta)$ .

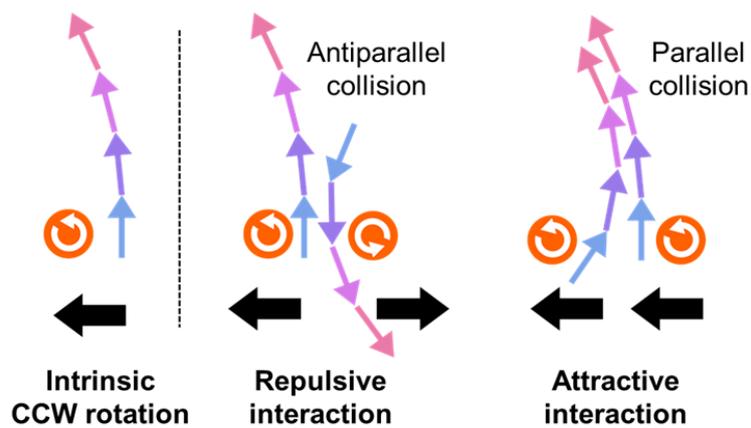
### Polarity analysis

Polarity analysis was performed from the fluorescence microscopy images in the following manner. Firstly, the flow fields, which should represent the microtubule motility, were obtained from the time-lapse images by the Gunnar-Farneback method of the optical flow. For this purpose, we employed the module ‘cv2.calcOpticalFlowFarneback’ in Python3. (Parameters are set as follows: flow=None, pyrScale=0.5, levels=2, winsize=15, iterations=3, polyN=5, polySigma=1.2, flags=0.) Next, we averaged the flow field both spatially and temporally. For the spatial average, we filtered each component of a flow vector on each pixel with the 201x201 averaging filter kernel. For the temporal average, we took the mean among consecutive 20 frames. Local polar order  $P$  is defined on each pixel ( $i$ ) at each frame as  $P(i) = |\mathbf{F}(i)|$  with such averaged flow vector  $\mathbf{F}(i)$  on the pixel. Then, the  $i$ -th pixel has been defined to be polarly ordered when  $P(i)$  is larger than a given  $P_{Cr}$ . We heuristically set this criterion to be  $P_{Cr} = 0.5$  by referring to the histogram of local polar order. Finally, we calculated the fraction of space covered by polarly ordered regions, which we termed “Polarity” and defined as the ratio of numbers of polarly-ordered and all pixels (Figure 2f). We used the first 50 frames of each movie for the case of chiral microtubules, whereas we used all the 31 frames for the case of the taxol-stabilized GDP microtubules.

## Supplementary figures



**Figure S1.** Polarity of microtubules in the periphery and center of the spiral pattern. (a) A fluorescence microscopy image shows bipolar phase at the periphery of a spiral pattern. Scale bar: 50  $\mu\text{m}$ . (b) Polarity of microtubules at the periphery and center of a large spiral pattern of microtubules.



**Figure S2.** Schematic representation shows monopolar alignment of microtubules mediated by interaction torque and intrinsic preferential CCW rotation of microtubules.

## Supplementary movie legends

**Movie S1.** Monopolar flocking of gliding chiral microtubules at the density of  $1.4 \times 10^5/\text{mm}^2$ .

Scale bar: 50  $\mu\text{m}$ .

**Movie S2.** Spiral pattern formation by microtubules at the density of  $2.4 \times 10^5/\text{mm}^2$ . Scale bar:

500  $\mu\text{m}$ .

**Movie S3.** Preferential rotation of microtubules to the CCW direction upon collision with the

micro-wall. Scale bar: 5  $\mu\text{m}$ .