Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Materials for

• Flagellar nanorobot with kinetic behavior investigation and 3D motion

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This file includes:

Supplementary Text:

Supplementary Figures:

Supplementary Tables

Other supplementary material for this manuscript includes the following:

Movie S1 (.mp4 format). Swimming of flagellar nanorobot in 2D through fluorescence microscopy imaging with different distances from the substrate.

Movie S2 (.mp4 format). Swimming of flagellar nanorobot in 2D through fluorescence microscopy imaging along the rectangular and "M" shapes.

Movie S3 (.mp4 format). Swimming of flagellar nanorobot in 2D through fluorescence microscopy imaging with different rotating frequencies.

Movie S4 (.mp4 format). Swimming of flagellar nanorobot in 2D through fluorescence microscopy imaging with different geometrical sizes.

Movie S5 (.mp4 format). Swimming of flagellar nanorobot in 2D through fluorescence microscopy imaging with different rotating directions.

Movie S6 (.mp4 format). Swimming of flagellar nanorobot in 3D through fluorescence microscopy imaging.

Movie S7 (.mp4 format). Swimming of flagellar nanorobot in 3D through bright-field microscopy imaging along the "S" "M" and "U" shapes.

Supplementary Text

Size measurement

By processing the image of the MH in bright-field with MATLAB, the area of the MH and the length of the scale bar in Figure S2 could be accurately obtained through correlation with the pixel size. Based on the length of the scale bar, the realistic length of each pixel edge represented could be calculated as well as the area of the MH. In this paper, the shape of the MH is assumed to be spherical and the influence of the polymorphism is ignored with flagella assuming normal form throughout. Therefore, the diameter of the MH could be calculated by the equation as follows:

$$D = \sqrt{\frac{4A}{\pi}} \tag{1}$$

where A is the area (μm^2) of the MH and D is the diameter of the MH.

Coordinates relationship

Figure 2B and C show the schematic of the rotating magnetic field, and the relative coordinate system (xyz) of the magnetic field based on the fixed reference ($X_cY_cZ_c$) shown in Eq. (2).

$$\boldsymbol{R}(\alpha,\beta,\gamma) = \boldsymbol{R}_x(\alpha)\cdot\boldsymbol{R}_y(\beta)\cdot\boldsymbol{R}_z(\gamma) \tag{2}$$

where $\boldsymbol{R}_x(\alpha) = \begin{vmatrix} 1 & 0 & 0 \\ 0 & \cos(\alpha) & -\sin(\alpha) \\ 0 & \sin(\alpha) & \cos(\alpha) \end{vmatrix}$, $\boldsymbol{R}_y(\beta) = \begin{vmatrix} \cos(\beta) & 0 & \sin(\beta) \\ 0 & 1 & 0 \\ -\sin(\beta) & 0 & \cos(\beta) \end{vmatrix}$ and $\boldsymbol{R}_z(\gamma) = \begin{vmatrix} \cos(\gamma) & -\sin(\gamma) & 0 \\ \sin(\gamma) & \cos(\gamma) & 0 \\ 0 & 0 & 1 \end{vmatrix}$; α, β and γ are the rotated angles of the x, y, and z axis about the X_c, Y_c and Z_c axis, respectively. The rotating magnetic field is then defined as

$$\boldsymbol{B} = \boldsymbol{R}(\alpha,\beta,\gamma)\cdot\left[B_x, 0, 0\right] \tag{3}$$

where the B_x is the set field magnitude aligned on the X_c axis. In this paper, the magnetic field magnitude is set as B_x=12 milliTesla (mT), in order to meet the requirement of working continuously for at least one hour without overheating issue.

Stokes' Law

The drag force *F* on a sphere of radius *r* moving through a fluid of viscosity η at velocity *v* is given by:

$$F = 6\pi r \eta v \tag{4}$$

Based on the Eq. (4), the drag force linearly increases as the *r* increasing.

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Supplementary Figures

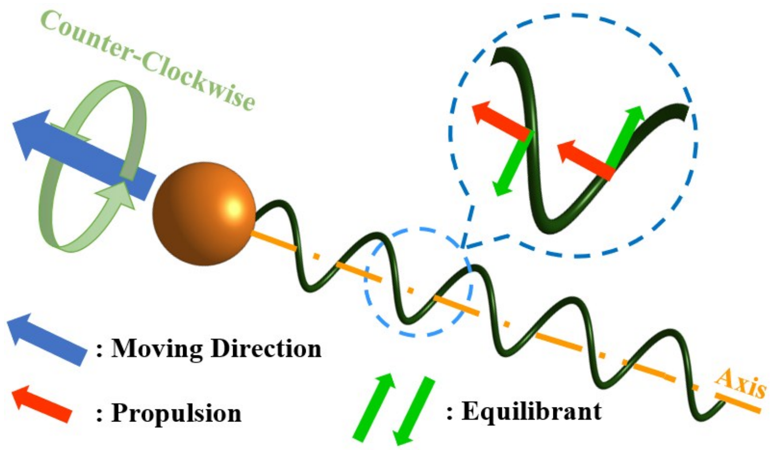

Figure S1. *Overall working mechanism of the flagellar nanorobot.*

Figure S2. *Overview of the magnetic field generator and fluorescence microscopy imaging system.*

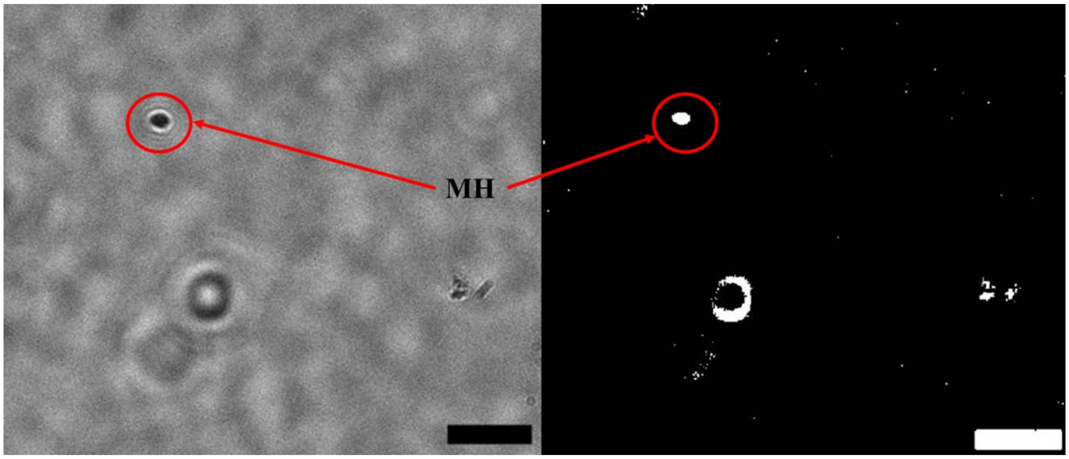

Figure S3. *Imaging of MH in bright-field before and after image processing. The scale bar is 10 µm. Other residual artifacts (other than the MH) have no meaning and were the result of the image binarization.*

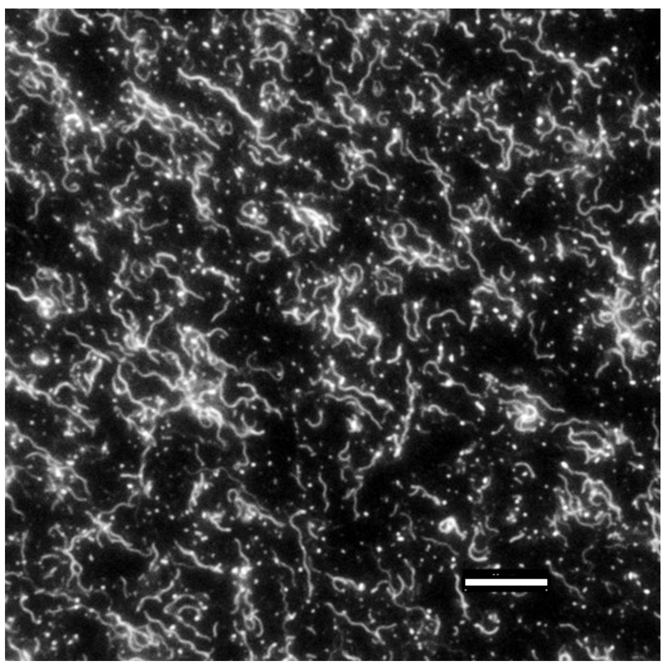

Figure S4. *Aggregated biotin repolymerized flagella which have different lengths in fluorescent field. The scale bar is 10 µm.*

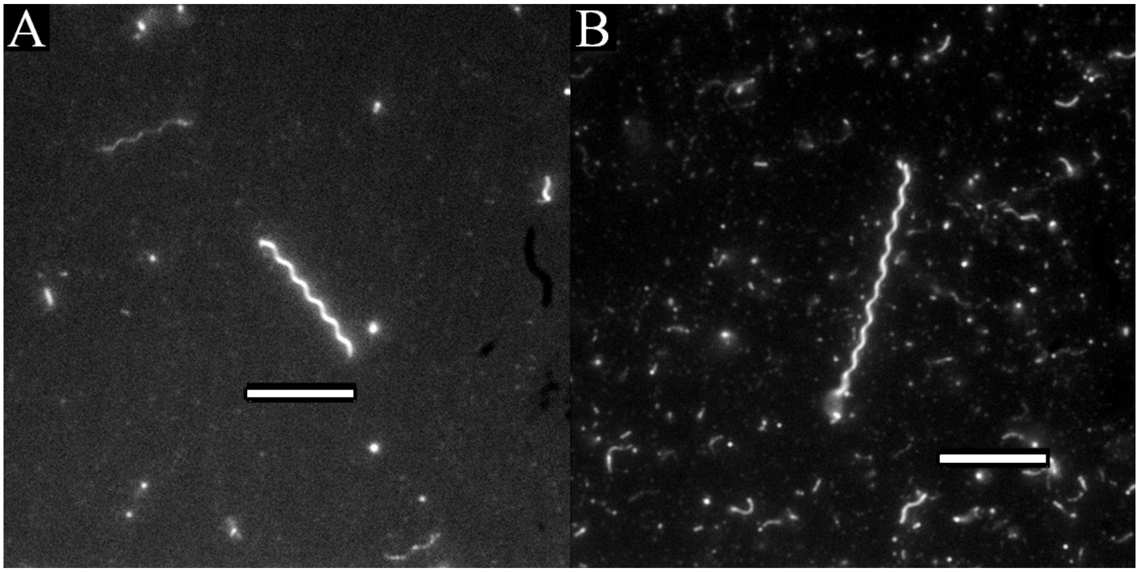

Figure S5. *Diluted biotin repolymerized flagella with different lengths. (**A**) 14.5 µm long biotin repolymerized flagellum. (**B**) 26.3 µm long biotin repolymerized flagellum. All scale bars are 10 µm*

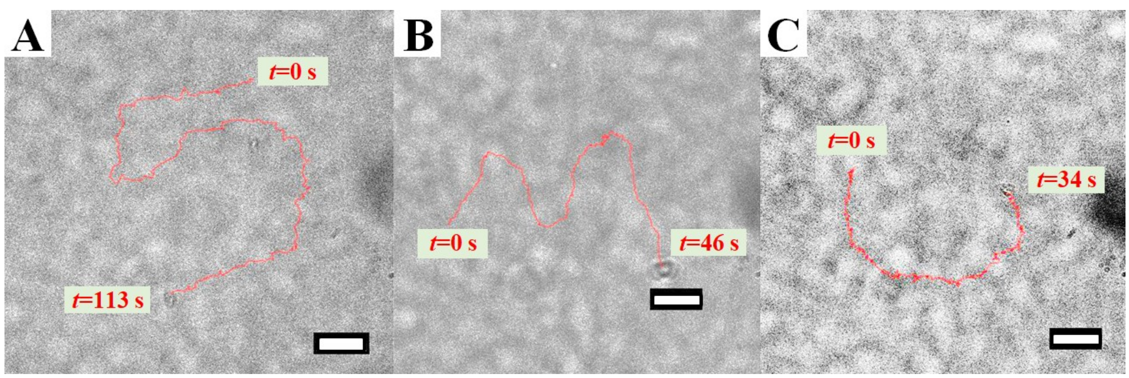

Figure S6. *Designed trajectories of the flagellar nanorobot under the magnetic control in the bright-field. (**A**) "S" shape trajectory of the flagellar nanorobot which is navigated by a 5 Hz rotating magnetic field. (**B**) "M" shape trajectory of the flagellar nanorobot which is navigated by a 5 Hz rotating magnetic field. (**C**) "U" shape trajectory of the flagellar nanorobot which is navigated by a 10 Hz rotating magnetic field. All scale bars are 10 µm.*

Supplementary Tables

Table S1. *The recipes of buffers in the flagella fabrication process*

Name	Ingredient	Notes
Modified Luria-Bertani (LB) broth recipe	1.00% Yeast extract 1.00% Tryptone 0.30% Glucose 0.66% K_2HPO_4 0.03% KH_2PO_4	In percentage by weight
Polymerization buffer	0.0034 M K_2HPO_4 0.0066 M KH_2PO_4 150 mM NaCl	In each liter of distilled water
Conjugation buffer	0.007 M K_2HPO_4 0.003 M KH_2PO_4 150 mM NaCl	In each liter of distilled water