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## **Supplementary Information**

## 3D Magnetically Controlled Spatiotemporal Probing and Actuation of Collagen Networks from a Single Cell Perspective

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Compund	Amount [per L]
Nickel sulfate hexahydrate	300 g
Nickel chloride hexahydrate	30 g
Cobalt sulfate hexahydrate	40 g
Citric acid anhydrous	40 g
Boric acid	20 g
Saccharin as ductilizer	2 g
TERGITOL 08 as wetting agent	2.5 mL

## Table S1. Preparation of the electrolyte solution for µRod synthesis

To prepare the electrolyte solution for  $\mu$ Rod synthesis, chemicals listed in Table S1 were combined and brought to solution in MQ water to a final volume of 1 L. Under magnetic stirring, the solution was heated to 50 – 55 °C and mixed until all components were dissolved. Using NaOH, the pH was adjusted to pH 5-7; the solution was filtered and stored at room temperature.

Sample		Lower (Percentile)	Upper (Percentile)	Wiener2 De-noise Span
20191202	1	0.01	99.8	5x5
	2	0.01	99.8	
	3	0.01	99.8	
20200128	2	0.01	99.95	
	3	0.01	99.95	
	4	0.01	99.95	5x5
	5	0.01	99.95	
	6	0.01	99.95	5x5
20200228	2	0.01	99.95	5x5
	3	0.01	99.95	5x5
	4	0.01	99.95	

## Table S2. Image thresholding parameters for fiber tracking

**Table S2** lists the image processing parameters used for tracking collagen fiber deflection. The lower and upper percentiles were used to threshold the image intensity. The Wiener2 MATLAB de-noising filter was used to revise images with a poor signal-to-noise ratio using the span listed. For visual representation, arrows generated by the MATLAB script were scaled up in length by a factor of 100.

## Fig. S1. µRod characterization



Fig. S1. Characterization of magnetic  $\mu$ Rods. (a) Size distribution of a sample of  $\mu$ Rods based on bright-field images. Average length: 5.9  $\mu$ m (± 1.7  $\mu$ m), average diameter: 1.3 (± 0.2  $\mu$ m); n=259 detected  $\mu$ Rods. Inset: Bright-field image of  $\mu$ Rods dispersed on a glass slide. Scale bar: 10  $\mu$ m. (b) Vibrating sample magnetometry measurement of  $\mu$ Rods dispersed in water. (c) EDX elemental surface analysis spectrum of a representative  $\mu$ Rod. Inset: Surface scan showing SEM image and elemental occurrence of Ni and Co. Additional peaks (C and Si) are attributable to the substrate and environmental contamination. Scale bars: 2  $\mu$ m.

# Fig. S2. µRod functionalization



20% ND-PEG-CF488A, 80% ND-PEG-NHS

**Fig. S2.** Functionalization schematic of CoNi  $\mu$ Rods. (a) Nitrodopamine was reacted with NHS-PEG-NHS (1:1 molar ratio) in dichloromethane overnight under nitrogen to obtain an ND-PEG-NHS linker for  $\mu$ Rod surface functionalization with NHS groups to allow for direct attachment to the collagen hydrogel matrices through NHS amidation. (b) Fluorescent  $\mu$ Rods were obtained by first preparing a ND-PEG-CF 488A stock solution. ND-PEG-NHS was reacted with CF 488A in 0.1 M triethylammonium acetate buffer. (c) ND-PEG-NHS (80%) and ND-PEG-CF 488A (20%) were pre-mixed and used to functionalize the  $\mu$ Rods. 20% labeling using ND-PEG-CF 488A was sufficient to enable imaging via fluorescence microscopy while maintaining efficient crosslinking to the collagen fiber network.



Fig. S3. Workflow to convert µRod deflection into values of shear modulus

**Fig. S3**. Flowchart showing decision tree for data processing of deflection data. Four examples of deflection versus time extracted from image processing are shown at the top. As a first step,  $\mu$ Rods that rotate more than 180° are identified, and bounds on rotational stiffness k are estimated.  $\mu$ Rods that rotate less than 180° are sorted on the basis of the periodicity they exhibit and fitted with one of two model scenarios: 1) one based on local energy minimization that assumes the moment stays locally pinned to the axis 2) another based on global energy minimization. The frequency domain characteristics differ for the two models, as shown.  $\mu$ Rods with very low deflection signals that do not reflect periodicity in the range of the applied rotating field frequency are excluded. The fitted parameters are the coefficients for the rotational stiffness function up to sixth order. Finally, the output of the finite element mechanical model relating rod length, shear modulus, and k to is used to define a numerical interpolation function that translates the fitted k<sub>2</sub> into an effective shear modulus.



120

30 60 90 Field Magnitude [mT]

#### Fig. S4. Sample-embedded µRod deflection analysis

Effective

120

30 60 90 Field Magnitude [mT]

1×10

Effective 1×10

Fig. S4. µRod deflection and modeled effective shear moduli as function of magnetic field magnitude and collagen concentration. Embedded µRod deflection was observed in Collagen I samples as a function of collagen concentration and magnitude of the applied magnetic field with an in-plane rotational frequency of 1 Hz. Three fields of view were analyzed per hydrogel sample, starting with the lowest field magnitude and increasing sequentially. Subsequent analysis of µRod deflection over time according to the decision tree in Fig. S3 determined effective shear moduli for each analyzed µRod. (a) Plot of deflection values extracted from measurements performed for collagen hydrogels at concentrations between 0.5 and 2 mg/mL. (b) Effective shear moduli computed from deflection values determined from µRods embedded in collagen. Blue triangles mark the lower bound of mean determined effective shear modulus values, yellow indicates the mean of the upper bound of determined effective shear moduli.

Effective

120

90 de [mT]

60 30 60 Field Magnit

120

30 60 90 Field Magnitude [mT]

### Fig. S5. Bulk rheological assessment of collagen hydrogels



*Fig. S5.* (*a*) Bulk shear rheology measurements of collagen I hydrogels tested at concentrations between 0.5 mg/mL to 2.0 mg/mL, n=5. Storage moduli are shown to increase with increasing collagen concentration, ranging between 5.7 Pa to 54.8 Pa for the tested concentrations. (b) Frequency sweep for collagen, n=5. Oscillatory frequency sweeps were performed from 1.0-10.0 Hz at 0.1% strain. The storage moduli for collagen hydrogels demonstrated strong frequency dependence across all concentrations. These results are consistent with previously published shear rheology results for 1 mg/mL collagen hydrogels.<sup>1</sup>

### Fig. S6. Stability of long-term actuation of collagen hydrogel



Fig. S6. Long-term actuation of  $\mu$ Rods embedded in collagen.  $\mu$ Rods embedded in 0.5 mg/mL collagen were continuously actuated by a 1.0 Hz 55 mT in-plane rotating field. Maximum deflection values monitored in 10 min intervals (a) and resulting values of effective shear modulus (b) indicate no significant change after 50 min actuation. A Mann Whitney test was used to test for significance. p = 0.2012 for max deflection (a) and p = 0.2703 for effective shear modulus values (b), n= 12-25  $\mu$ Rods.





**Fig. S7.** Analysis of TAMRA-labeled collagen, testing for the introduction of staining induced structural artifacts and CT fire analysis. 0.5 mg/mL collagen hydrogels were prepared with and without TAMRA-labeling to compare potential effects of TAMRA-labeling on hydrogel structure. Hydrogels were imaged using second harmonic generation (a-b) Scale bars: 50  $\mu$ m (left a and b), 20  $\mu$ m (right a and b). The CT-FIRE algorithm was used to compare fiber length (c), straightness (d), width (e), and angle (f-g). Overall the two collagen hydrogels exhibited similar structures, and there was no significant difference between the two conditions based on any of the metrics observed. Analysis via Student's t-test shows significance with p>0.05.

## Fig. S8. Local deformation of collagen network



**Fig. S8.** Local deformation of a TAMRA-labeled collagen network. Time series of confocal fluorescence micrographs of clockwise  $\mu$ Rod deflection over one period at 73 mT and 0.1 Hz frequency.  $\mu$ Rod position and orientation is extracted from the respective bright field micrographs (see insets) and is marked by the white dashed line. Time points correspond to data presented in Fig. 4. For original video, see Video S2. All scale bars: 10  $\mu$ m.

## Fig. S9. Cell viability test



**Fig. S9.** Evaluation of cell viability by MTT assay. Human Foreskin Fibroblasts were cultured in the presence of different  $\mu$ Rod concentrations and tested for viability over three days. Ctrl: No treatment; TEA Ctrl: 0.1 M triethylammonium acetate buffer without  $\mu$ Rods; Low Rod Conc.: Concentration of  $\mu$ Rods applied in 3D culture experiments shown in Fig 4e-f; High Rod Conc.: Ten-fold concentration of  $\mu$ Rods applied in 3D culture experiments shown in Fig 4e-f. (n=3).



**Fig. S10.** (a) Photograph of the electromagnetic field generator Magnebotix MFG-100-i with core extensions. The picture depicts insertion of core extensions into the electromagnetic field generator. The inset shows details of the core extensions facing the sample. (b) The workspace of the electromagnetic field generator was calibrated using a Metrolab 3D magnetometer. Controlled by a piezo-guided micromanipulator, the magnetic probe was used to scan the workspace. With the core extensions in place, the working volume of 1 mm<sup>3</sup> was scanned in volume steps of 100  $\mu$ m (x/y/z), performing 32 measurements per point at an applied current of 2 A. (b) Vector plots of field generated by each coil are shown and (c) a performance evaluation shows percent variation in the x, y, and z directions, as well as variation in vector magnitude.

#### Supplementary Section 1. Detailed explanation of the physical model

We consider a magnetic  $\mu$ Rod entrapped in an elastically deformable matrix. Let us consider it uniformly magnetized with moment  $\vec{m}$ . (Note: Though this assumption will not be well motivated during the instant of magnetization reversal, it reasonably describes the equilibrium states of the  $\mu$ Rod in an applied field) A field  $\vec{H}$  will be rotated within in a single plane, and resulting angular displacements are assumed to occur within that plane. A schematic display of this system is shown in Fig. SS1.1.



Fig. SS1.1. Sketch of quantities defined for the physical model of a  $\mu$ Rod embedded in an elastic matrix.  $\alpha$  is displacement of the long axis from mechanical equilibrium.  $\gamma$  is the angular orientation of the moment of particle m.  $\theta$  is the orientation of the applied field H.

Let  $\alpha$  be the angular displacement of the  $\mu$ Rod from its mechanical equilibrium state. Some function  $U_{mech}(\alpha)$  describes the energy penalty of rotating the  $\mu$ Rod away from this equilibrium. Anticipating the symmetry of mechanical forces upon displacement in either direction from equilibrium, the unknown functional form of the  $U_{mech}(\alpha)$  can be expressed in terms of a Taylor series expansion about  $\alpha = 0$ , as follows:

$$U_{mech}(\alpha) = \frac{1}{2!}k_2\alpha^2 + \frac{1}{4!}k_4\alpha^4 + \frac{1}{6!}k_6\alpha^6 + \dots$$
(1)

Here,  $k_n$  is the coefficient for the n<sup>th</sup> term in the expansion. Although we allowed  $k_4$  and  $k_6$  to vary in our fitting algorithm,  $k_2$  was used to find effective shear modulus. In the case that higher order terms vanish, or in the limit of small angular displacements as  $\alpha \rightarrow 0$ , this reduces to a rotational version of Hooke's Law:

(2)

$$U_{mech}(\alpha) \approx \frac{1}{2}k_2\alpha^2.$$

Here,  $k_2$  is the "rotational stiffness." Relating  $k_2$  back to effective mechanical properties of the matrix depends not just on the mechanical properties of the surrounding material, but also the geometry of the  $\mu$ Rod, as discussed later in this section.

The long axis of the  $\mu$ Rod coincides with its preferred direction of magnetization. This allows us to write the energetic contribution of magnetic shape anisotropy in terms of the angular displacement between the long axis of the  $\mu$ Rod and its magnetization vector,  $\gamma - \alpha$ . In general, the energetic contribution arising from shape anisotropy energy can be given by

$$U_{ani}(\hat{m}) = \frac{M_s^2 \mu_0 V}{2} \left( N_x m_x^2 + N_y m_y^2 + N_z m_z^2 \right),$$
(3)

where  $\hat{m}$  is a unit vector with components  $(m_{x}, m_{y}, m_{z})$  expressing the direction of the moment relative to the coordinate system aligned along the principal axes of the demagnetizing tensor.<sup>2</sup>  $M_{s}$  is the magnetization of the material comprising the  $\mu$ Rod, V is the volume, and  $\mu_{0}$  is the permeability of free space. In the case of a cylinder of finite length, the z axis is usually taken to correspond to the axis of the cylinder, such that

$$N_x = N_y = (1 - N_z)/2.$$
(4)

For cylinders,  $N_z$  has both exactly calculated values and approximate forms that are offered in literature as a function of the aspect ratio. Substituting Equation 4 into Equation 3 and recasting in terms of variables defined for the model,

$$U_{ani}(\gamma,\alpha) = \frac{M_s^2 \mu_0 V}{2} \left[ \frac{1 - N_z}{2} \sin^2(\gamma - \alpha) + N_z \cos^2(\gamma - \alpha) \right].$$
 (5)

We can use a trigonometric identity to find an alternative expression:

$$U_{ani}(\gamma,\alpha) = \frac{M_s^2 \mu_0 V}{2} \left[ \frac{1 - 3N_z}{2} \sin^2(\gamma - \alpha) + N_z \sin^2(\gamma - \alpha) + N_z \cos^2(\gamma - \alpha) \right]$$
(6)

$$=\frac{M_{s}^{2}\mu_{0}V}{2}\left[\frac{1-3N_{z}}{2}\sin^{2}\left(\gamma-\alpha\right)+N_{z}\right].$$
(7)

Only the terms with angular dependence on  $\gamma$  or  $\alpha$  will result in physical torque because  $N_z$  is fixed by geometry and just adds a constant. Nevertheless, we retain it in the model.



Fig. SS1.2. Anisotropy energy as a function of  $\gamma$  is shown for  $\alpha = 0$  for various aspect ratios of hypothetical cylindrical  $\mu$ Rods of fixed volume (2.5 $\pi$   $\mu$ m<sup>3</sup>). The lowest aspect ratios describe discs, whereas the highest ones approach values relevant for our  $\mu$ Rods.

In the limit  $N_z \rightarrow 0$ , which is to say in the limit of a very long cylinder, one can show how the expression can be recast in terms of  $\cos^2(\gamma - \alpha)$  and is equivalent to a simplified version, up to an added constant. Re-expressing Equation 7 with the Pythagorean identity,

$$\frac{M_s^2 \mu_0 V}{2} \left[ \frac{1}{2} \sin^2 (\gamma - \alpha) \right] = \frac{M_s^2 \mu_0 V_1}{2 2} \left[ 1 - \cos^2 (\gamma - \alpha) \right].$$
(8)

Up to an added constant (unimportant when considering torques or minimizing energy) this approximate form can be given by

$$U_{ani}(\gamma,\alpha) \approx -\frac{M_s^2 V \mu_0}{4} \cos^2{(\gamma - \alpha)}.$$
(9)

Finally, the interaction of the moment of the  $\mu$ Rod with the applied field contributes an additional energy term  $U_{field}$ . Maintaining the assumption of approximately uniform magnetization of the  $\mu$ Rod, this can be expressed as

$$U_{field}(\gamma,\theta) = -M_s V \mu_0 H \cos\left(\theta - \gamma\right). \tag{10}$$

*H* is the magnitude of the applied field. Summed together, the contributions to the energy of the system yield

$$U(\gamma, \alpha, \theta) = U_{mech}(\alpha) + U_{ani}(\gamma, \alpha) + U_{field}(\gamma, \theta)$$
(11)

In the experiments being described,  $\theta$  is varied continuously over its full range of possible values, variation in  $\alpha$  is observed with a microscope, and  $\gamma$  varies but is not directly observable. When a particular field orientation is applied, the  $\mu$ Rod relaxes to some overall equilibrium state with a particular  $\gamma$  and  $\alpha$ . To relate the rotational stiffness to the behavior of the system, we need to study how  $\alpha$  varies with  $\theta$ . In principle, we could approach the minimization of the

equation for energy above analytically, setting  $\overline{\partial \alpha}$  and  $\overline{\partial \gamma}$  to zero separately and solving the set of simultaneous equations for  $\alpha$  and  $\gamma$ . However, these functions have multiple local minima and given the large datasets produced through image analysis, it was convenient to minimize numerically.

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дU

Two algorithms were implemented, one that assumes a global energy minimum is reached (i.e. the system is not kinetically limited) and one that tracks the evolution of a local minimum. The predicted behavior is sufficiently different that comparison to data straightforwardly reveals which assumption is most appropriate, and there are instances of each in our data. (See Fig. S3).

As a generalized examination of the behaviors predicted by the model, we assumed a Ni-Co  $\mu$ Rod with a composition matching the one determined by EDX, and a hypothetical 1  $\mu$ m

diameter, 10  $\mu$ m length, and 15 mT rotating field magnitude. In this preliminary test, rotational stiffness was varied with values selected on the basis of illustrating different behaviors observed in experiments with  $\mu$ Rods. The results are summarized in Fig. SS1.3.



**Fig. SS1.3.** Expected deflection behavior for a range of rotational stiffness values is considered with algorithms based on global (a) or local (b) energy minimization.

The global minimum deflection curves display a consistent symmetry and presence of two jump discontinuities regardless of the order of magnitude of the stiffness. In contrast, for stiff matrices, the local minimum deflection curves do not exhibit jump discontinuities. For matrices that are less stiff, orientation tracks with the applied field for a greater fraction of its rotation than the global minima curves, and eventually exhibit a jump discontinuity when the local minimum vanishes. In this simple model, damping is not included, but its influence would be most significant in the vicinity of these jumps.

As noted in Equation 1, with large deflections and with nonlinear media, it is not reasonable to expect the mechanical energy to scale quadratically with the angular displacement. The higher order terms are needed to describe behavior arising from effective "stiffening" encountered with large deflections. To get a sense for how these terms affect the model, we considered cases of each term acting in isolation in the local minimum model. One is in the "continuous deflection" regime where material is stiff enough with  $k_2$  to prevent large deformation and snap back. The other assumes a ten times softer matrix, such that a discontinuity is expected. These results are shown in Fig. SS1.4.



**Fig. SS1.4.** Expected deflection behavior considering the influence of  $k_2$ ,  $k_4$ , and  $k_6$ . Holding all else constant with Fig. SS1.3, the influence of these higher order stiffness terms is shown for local energy minimization with continuous deflection (a) and discontinuous deflection (b).

The  $k_4$  and  $k_6$  cases permit much higher initial displacement, essentially not resisting the torque applied by the field, but ultimately jump back at the onset of resistance to further deflection. Even if  $k_4$  and  $k_6$  assume comparable or larger values than  $k_2$ , the effective shear modulus would be extracted from  $k_2$  given that it dominates  $U_{mech}$  at small displacements. Inferring effective shear modulus from small displacements of a cylinder in an elastic matrix with known applied torques has previously been treated analytically,<sup>3</sup> though this approach is only applicable to small angular deflections where geometric nonlinearities can be neglected. However, the range of deflection values observed in this study precludes this assumption. To address this limitation, a finite element (FE) model was established in COMSOL Multiphysics to numerically derive a function correlating effective shear modulus with the second order term of the rotational stiffness, accounting for geometric characteristics of the  $\mu$ Rods.

The mechanical model was comprised of a rigid  $\mu$ Rod surrounded by linear elastic material. The effect of geometric nonlinearities was included in the governing solid mechanics equations. A rigid dipole moment was assigned to the  $\mu$ Rod aligned with its major axis. Magnetic torque exerted on the  $\mu$ Rod was calculated as follows

$$T = mBsin\theta \tag{12}$$

Where *m* represents the magnetic moment of the  $\mu$ Rod calculated based on the saturation magnetization of nickel and cobalt given the ratio obtained from EDX. B is the magnetic field which rotates at 1 Hz, and  $\theta$  indicates the phase lag between these two vectors.

Simulations were conducted for 2 s to ensure that the effect of inertia was negligible by comparing the first two cycles of actuation. To incorporate the effect of local densification, a mathematical expression for shear modulus was defined as a function of distance from surface of the  $\mu$ Rod. The size of the densified area was extracted from fluorescent microscopy images and was set to 15  $\mu$ m for the FE model. The influence of a nearby rigid boundary surface was also studied by positioning the  $\mu$ Rod at different distances from a boundary while keeping the rest of boundaries far away from the  $\mu$ Rod.

Resulting angular deflections of the  $\mu$ Rod located in a material with bulk shear modulus G = 1 kPa at 100 mT under different conditions illustrate the contribution of both effects to the behaviour of the actuated  $\mu$ Rod (Fig. SS1.5).



**Fig. SS1.5.** Angular deflections of a  $\mu$ Rod positioned at different heights relative to the bottom wall inside an elastic material with 1 kPa shear modulus. Displacements are larger in the absence of local stiffening (a) compared to the rods experiencing 2-fold (c) and 10-fold (c) localized increase in the material stiffness at the surface.

Next, rotational stiffness of the material at different  $\mu$ Rod lengths and shear moduli was extracted by using the first term of Equation 1 (Fig. SS1.1). This numerically derived matrix was used to estimate the effective shear modulus based on  $k_2$  values obtained from the fitting algorithm for experimental datapoints. As a cross validation between the two computational models, angular deflections from FE simulations were fed into the fitting algorithm (Fig. SS1.6). Comparing the estimated effective modulus with the value assumed for the elastic material in simulations reveals approximately 20% difference. This deviation was mainly attributed to the assumptions behind each modeling approach. Higher order *k* terms are neglected when extracting  $k_2$  from mechanical strain energy in FE model, plus the rigid dipole moment assumption is not perfectly valid under all conditions as demonstrated by the fitting algorithm.



**Fig. SS1.6.** Cross validation between two models. (a) Matrix correlating shear modulus with rotational stiffness as a function of  $\mu$ Rod length. (b) Calculated shear moduli from the fitting algorithm fed with angular displacements from the FE simulations under prescribed bulk shear modulus.

#### Supplementary Section 2. Image-based tracking analysis

#### Text S2.1. Tracking µRod actuation in 2D

Bright-field image sequences of  $\mu$ Rods were captured during exposure to magnetic actuation. Post-processing included inversion, blurring, binarization, and adaptive thresholding to eliminate imaging-induced brightness artifacts. Concluded by erosion and dilation, this first step identified  $\mu$ Rod candidates while removing as much background as possible.<sup>4</sup> Subsequent in-depth analysis of the previously identified  $\mu$ Rod candidates relied on MATLAB's "regionsprops" algorithm and provided information including position, morphology (size, length, and width), solidity and orientation. Analyzed regions were then filtered for rod-like appearance based on width, aspect ratio, and solidity to exclude false signals.

In a final refinement stage, previously determined candidate  $\mu$ Rods were revisited, and a more detailed analysis of dimensions was performed. First, the orientation was determined by minimizing the distance of the centerline to the edge pixels of the  $\mu$ Rod using the Nelder-Mead algorithm. Applying a threshold to a normalized intensity profile along the centerline, each  $\mu$ Rod's axis was identified, and the center was defined as the position of the respective  $\mu$ Rod. Comparing the occupied area of the  $\mu$ Rod to the area occupied by a rectangle with identical dimensions, debris, or defective  $\mu$ Rods were excluded from further analysis.

To monitor individual  $\mu$ Rods throughout an image sequence, a virtual  $\mu$ Rod was created for every suitable  $\mu$ Rod candidate detected in the first frame. A  $\mu$ Rod identified in frame  $f_{t+1}$  with centroid position  $p_{t+1}^{x}$  was linked to a  $\mu$ Rod in the previous frame  $f_t$  with centroid position  $p_t^{A}$ if the distance between the two centroids  $\|p_t^A - p_{t+1}^x\|$  was smaller than the distance to any other  $\mu$ Rod's centroids position in frame  $f_t$  and did not exceed a pre-defined threshold. In this manner,  $\mu$ Rod characteristics of every frame could be assigned to a respective virtual  $\mu$ Rod and analyzed as a function of time.

#### Text S2.2 Tracking of collagen fiber actuation

NHS-functionalized  $\mu$ Rods were embedded within 0.5 mg/mL TAMRA-labeled collagen hydrogels and actuated at 0.1 Hz and 73 mT in the XY-plane. Imaging was performed using a Nikon Eclipse Ti2 microscope with a Yokogawa CSU-W1 confocal scanner unit, Nikon NIS Elements software, a Hamamatsu C13440-20CU ORCA-Flash4.0 V3 digital CMOS camera, and a Nikon CFI Plan Apochromat  $\lambda$  40X short working distance (250 µm) objective with 0.95 NA, and a pixel-to-distance ratio of 0.16 µm<sup>2</sup> per pixel.

The hydrogel actuation was analyzed using MATLAB by quantifying the area of influence

(AOI) and the mean deflection velocity of the ECM. The AOI of a rotating  $\mu$ Rod was determined from image series acquired during rotating actuation of the  $\mu$ Rod. First, the images were post-processed to improve contrast using an Autoscale function that scales the intensity distribution based on a pre-defined low and high threshold limit. Values for the Autoscale percentiles can be found in Table S2. All pixels with intensity values outside of the low-high input range were set to the maximum or minimum values, and the remaining pixels were scaled to fill the full range. If necessary, a 2D adaptive noise-removal filter (wiener2) was applied with a 5x5 pixel span.

To characterize the velocity of collagen fiber displacement, images of the video sequence were analyzed for optical flow, which describes the perceived relative motion during each timestep. The optical flow between images in a time series was estimated by a Horn-Schunk tracker (opticalFlowHS) with Smoothness = 0.1, MaxIteration = 100, and VelocityDifference = 0. The tracker returns a magnitude and direction of the flow at every pixel. A map of flow magnitudes was created and the values from the area occupied by the  $\mu$ Rod was discarded. The map of these flow magnitudes was then filtered with a 2D Gaussian kernel, thresholded with  $\sigma$  = 90 and binarized using the function "imbinarize". Next, 8-connected objects were identified to belong to the same physical structure. The pixels in these regions were grouped at their magnitude and orientation. Arrows were used to visualize these groups. To aid visualization, the magnitudes of the arrows indicating mean deflection velocity were multiplied by a factor of 100.

To eliminate the effect of µRods outside the field of view, only AOIs with a centroid inside a 200x200-pixel box in the center of the image were considered. Similarly, only arrows located within the diameter of the AOI were considered. Next, the functions of AOI and mean deflection velocity over time were smoothed using a span of 3, and the local maxima with prominences equal to the 25% of the max-min range of values were determined. In the case where local maxima were not detected, the maximum value for AOI or mean deflection velocity was used for that sample. Finally, the mean radius of influence was calculated from the peak AOI values. The Matlab code for the described fiber displacement analysis is made available in Text S2.4.

#### Text S2.3 Tracking of µRod actuation in 3D

Fluorescently labeled  $\mu$ Rods were embedded in 0.5 mg/mL TAMRA-labeled collagen matrices and imaged using confocal microscopy. The magnetic field orientation was visually aligned with the orientation of the resting  $\mu$ Rod based on bright-field imaging. The field strength was ramped up from 0 mT to 73 mT in the same orientation. The magnetic field was then rotated clockwise to induce  $\mu$ Rod deflection up to the point of maximum deflection. 10-15  $\mu$ m image stacks were acquired with 0.16  $\mu$ m step size to ensure cubic voxels.

Fluorescently labelled  $\mu$ Rods were detected with a hybrid approach using bright-field and fluorescent image stacks to extract 3D  $\mu$ Rod poses. Using this method, even  $\mu$ Rods with incomplete fluorescence could be reliably detected. Briefly, the bright-field image stack was inverted, thresholded, eroded, and dilated. The fluorescent image stack was blurred, thresholded, eroded, and dilated. The resulting two binary tensors were combined with a logical AND operation, selecting for regions detected in both bright-field and fluorescence.

Analogous to the 2D  $\mu$ Rod pose extraction, MATLAB's "regionprops3" function was used to extract information about the connected regions in the post processed image stack. Orientation was calculated as the normal vector collinear with the longitudinal axis of the region. The regions were filtered according to volume, solidity, aspect ratio, and length of the two short principal axes. Next, the largest region and its corresponding image were compared with an ideal  $\mu$ Rod of identical orientation and length. If the occupied volume was considered similar enough based on previously defined parameters, the  $\mu$ Rod was kept. Finally, maximum deflection angles were calculated from the difference between the  $\mu$ Rod orientation vectors.

Confocal fluorescence image stacks of the CF488-labeled  $\mu$ Rods were improved using Bitplane Imaris to remove background noise and hot pixels from the microscope camera. Briefly, a surface was created around the fluorescent  $\mu$ Rod using the Imaris Surfaces tool, and the entire green channel was masked. This converted all values outside the surface to zero signal. To improve the visualization of the TAMRA-labeled collagen structure, the confocal image stacks were deconvolved using the Huygens Remote Manager v3.5.

### Text S2.4 Code for tracking of collagen fibers

The following code has been written in Matlab for the analysis of collagen fiber displament

```
tic
clear all
close all
dirname = ''; %Enter directory
ext = '.nd2';
listing = dir(dirname);
filenames = {};
%OPTIMIZED VARIABLES
PrctMin=0.01; %Lower percentile limit for Autoscale
PrctMax=99.98; %Upper percentile limit for Autoscale
Denoise=0; %Denoising span
longer = 100; %Multiplication for display of arrows (in image only)
Smooth=3; %Smoothing span for AOI and arrow plots for peak detection
for i=1:size(listing,1)
    if size(listing(i).name,2) > 4
        if strcmp(listing(i).name(end-size(ext,2)+1:end), ext)
            filenames{end+1} = listing(i).name;
        end
    end
end
mkdir(dirname, 'tifs');
data = bfopen([dirname, filenames{1}]);
tamramap = data\{1, 3\}\{1, 1\};
%% read in frames
for e=1:size(filenames,2)
    disp('Reading Frames');
    vidname = filenames{e};
    88
    vidpath = [dirname , vidname];
    reader = bfGetReader(vidpath);
    omeMeta = reader.getMetadataStore();
    planeCount = reader.getImageCount();
    voxelSizeX =
double(omeMeta.getPixelsPhysicalSizeX(0).value(ome.units.UNITS.MICRO
METER)); % in um
    channelCount = omeMeta.getChannelCount(0);
    channelName = [];
    um per px = voxelSizeX;
    for i=1:channelCount
        channelName{i} = string(omeMeta.getChannelName(0,i-1));
    end
    desChannel = 0;
```

```
for i=1:channelCount
        if strcmp(channelName{i}, 'TxRed W1')
            desChannel = i;
        end
    end
    for i=1:planeCount/channelCount
        stack{i} = bfGetPlane(reader, i*desChannel);
        disp(num2str(100*i/(planeCount/channelCount)));
    end
    %% track optical flow
    toc
    disp('Tracking Optical Flow');
    opticFlow1 =
opticalFlowLKDoG('NumFrames',3,'ImageFilterSigma',3.5,'GradientFilte
rSigma',1,'NoiseThreshold',0.003);
    opticFlow2 =
opticalFlowHS('Smoothness',1,'MaxIteration',inf,'VelocityDifference',0
.001);
    opticFlow3 =
opticalFlowHS('Smoothness',0.1, 'MaxIteration',100, 'VelocityDifferenc
e',0);
    opticFlow4 = opticalFlowLK('NoiseThreshold', 0.003);
    close all
    h = figure;
    movegui(h);
    hViewPanel = uipanel(h, 'Position', [0 0 1 1], 'Title', 'Plot of
Optical Flow Vectors');
    hPlot = axes(hViewPanel);
    flowhist1 = [];
    flowhist2 = [];
    flowhist3 = [];
    flowhist4 = [];
    imarr = [];
    planejump = 5;
    imind = 1;
    for i=1:planejump:planeCount/channelCount
        im = im2double(stack{i});
        if i == 1
            med v = median(im(:));
        end
        Min=0;
        Max=prctile(im(:),PrctMax);
        if Denoise>0
            imarr{imind} =
wiener2(rescale(im, 'InputMin', Min, 'InputMax', Max), [Denoise, Denoise])
;
        else
```

```
imarr{imind} =
rescale(im, 'InputMin', Min, 'InputMax', Max);
        end
        flow3 = estimateFlow(opticFlow3,imarr{imind}); %Estimate
optical flow between consecutive video frames
        flowhist3 = [flowhist3, flow3];
        imind = imind+1;
        Progress=(i/planeCount)*100;
        disp(num2str(Progress));
    end
    %% analyze flow
    toc
    disp('Analyzing Flow');
    flowhist = flowhist3;
    figure('units', 'normalized', 'outerposition', [0 0 1 1])
    for i = 1:size(flowhist,2)-1
        figure
        movim = flowhist(i).Magnitude;
        movim = imgaussfilt(movim,90); %filter image movim with 2D
Gaussian smoothing kernel, stdev is set to 90, smoothens
        movim = rescale(movim); %rescale entries of movim to
interval between 0 and 1.
        bars = 250;
        bars = 0;
        movim(1:bars,:) = 0;
        movim(end-bars:end,:) = 0;
        movim(:, 1: bars) = 0;
        movim(:,end-bars:end) = 0;
        movimbin = imbinarize(movim); %binarization, default is
Otsu's method that aims to minimize interclass variance of
thresholded black and white pixels.
        imshow(movimbin);
        magnitude = flowhist(i).Magnitude;
         imshow(flowhist(i).Magnitude)
        rodmask = ~imbinarize(rescale(imarr{i}));
        rodmask = imerode(rodmask, strel('diamond',3));
        imshow(rodmask);
        magnitude = magnitude.*rodmask;
        imshow(magnitude,[])
```

```
vx = flowhist(i).Vx.*movimbin.*rodmask;
        vy = flowhist(i).Vy.*movimbin.*rodmask;
        %%identify pixels that belong to the same object:
        magnitudebin = bwlabel(imbinarize(magnitude)); %bwlabel
generates label matrix containing labels for 8-connected objects
found in imbinarize (which binarizes the image)
        statsmag = regionprops(magnitudebin, mat2gray(magnitude),
'PixelIdxList', 'MeanIntensity', 'WeightedCentroid', 'Area',
'MaxFeretProperties', 'MinFeretProperties'); %Properties of the area
that is given by connected pixels.
        movimblob = regionprops(movimbin, 'Centroid', 'Area',
'MaxFeretProperties', 'MinFeretProperties');
        consmag = zeros(size(magnitude));
        consvx = zeros(size(magnitude));
        consvy = zeros(size(magnitude));
        for k = 1:size(statsmag,1)
consmag(round(statsmag(k).WeightedCentroid(1)),round(statsmag(k).Wei
ghtedCentroid(2))) = statsmag(k).MeanIntensity; %(1) and (2)
indicate the x and y coordinate
consvx(round(statsmag(k).WeightedCentroid(1)),round(statsmag(k).Weig
htedCentroid(2))) = mean(vx(statsmag(k).PixelIdxList));
consvy(round(statsmag(k).WeightedCentroid(1)),round(statsmag(k).Weig
htedCentroid(2))) = mean(vy(statsmag(k).PixelIdxList));
        end
        [row,col,consvx_ind] = find(consvx);
        [~,~,consvy ind] = find(consvy);
        movimbin = rescale(movimbin);
        movimedge = edge(movimbin);
        movimedge dil = imdilate(movimedge, strel('diamond',2));
        color1 = [0 255 0]/255; %Color for AOI
        color2 = [0 0 255]/255; %Color for Arrows
        imshow(rescale(imarr{i}))
        imshow(imarr{i},tamramap)
        colormap(tamramap);
        imagesc(imarr{i});
        hold on
        green = cat(3, color1(1)*ones(size(rescale(imarr{i}))),
color1(2)*ones(size(rescale(imarr{i}))),
color1(3)*ones(size(rescale(imarr{i}))));
```

```
h = imshow(green);
        set(h, 'AlphaData', rescale(movimedge_dil))
        max len = max(consmag(:));
        arrows.xpos = [];
        arrows.ypos = [];
        arrows.xvel = [];
        arrows.yvel = [];
        if ~isempty(max len)
            %longer = 300/max len;
            for j=1:size(row,1)
                arrows.xpos = [col(j); arrows.xpos];
                arrows.ypos = [row(j); arrows.ypos];
                arrows.xvel = [consvx_ind(j); arrows.xvel];
                arrows.yvel = [consvy_ind(j); arrows.yvel];
                quiver(row(j), col(j), -longer*consvy ind(j), -
longer*consvx ind(j),'LineWidth',1,'Color',color2,'MaxHeadSize',0.3)
;
            end
        end
        hold off
        drawnow();
        F(i) = getframe(gcf) ;
        info(i).area=[movimblob.Area].*um_per_px^2;
        info(i).centroid=[movimblob.Centroid]; %position in pixels
        info(i).min diam=[movimblob.MinFeretDiameter].*um per px;
        info(i).max diam=[movimblob.MaxFeretDiameter].*um per px;
        info(i).arrows = arrows;
        Progress=(i/(size(flowhist,2)-1))*100;
        disp(num2str(Progress));
    end
    toc
    writerObj = VideoWriter('myVideo.avi');
    writerObj.Quality = 100;
    writerObj.FrameRate = 5;
    % set the seconds per image
    % open the video writer
    open(writerObj);
    % write the frames to the video
    disp('Writing Video');
    for i=2:length(F)
        % convert the image to a frame
        frame = F(i);
        writeVideo(writerObj, frame);
    end
    % close the writer object
    close(writerObj);
    응응
end
```

```
imwrite(rescale(im,'InputMin',Min,'InputMax',Max),'imarr rescale.jpg
');
imwrite(wiener2(rescale(im, 'InputMin', Min, 'InputMax', Max), [5,5]), 'im
arr wiener2.jpg');
        imwrite(rodmask, 'rodmask.jpg');
        imwrite(magnitude, 'magnitude.jpg');
        imwrite(F(i).cdata, 'AOIArrows.jpg');
save('info.mat','info');
disp('Closing Images');
close all;
toc
%clear all;
disp('Filtering AOIs & Velocities');
CentroidBox=200;
Size=size(info);
for i=1:Size(2)
    AreaSize=size(info(i).centroid);
    Data(1, 1) = 0.3;
    Data(1+i,1)=Data(i,1)+0.25;
    for k=1:AreaSize(2)/2
        if (512-CentroidBox<info(i).centroid(2*k-1)) &&
(info(i).centroid(2*k-1)<=512+CentroidBox) && (512-
CentroidBox<info(i).centroid(2*k)) &&</pre>
(info(i).centroid(2*k)<=512+CentroidBox)</pre>
        Data(i, 2) = info(i) . area(k);
            for j=1:numel(info(i).arrows.xvel)
                 ypos=info(i).arrows.ypos(j);
                 centy=info(i).centroid(2*k);
                 xpos=info(i).arrows.xpos(j);
                 centx=info(i).centroid(2*k-1);
                 diam=info(i).max diam(k)/0.16;
                 if sqrt((ypos-centy)^2+(xpos-centx)^2)<diam;</pre>
ArrowMag(j+2,i)=sqrt((info(i).arrows.xvel(j))^2+((info(i).arrows.yve
l(j))^2));
Data(i,3)=sqrt((info(i).arrows.xvel(j))^2+((info(i).arrows.yvel(j))^
2));
                 end
            end
        end
    end
    for j=1:numel(info(i).arrows.xvel)
    end
end
%Finding AOI & Vel Peaks
```

```
A(:,1)=Data(1:end,2);
A(:,2)=Data(1:end,3);
Data(1:end, 4:5) =A;
[PeaksArea(:,2),PeaksArea(:,1),w,PeaksArea(:,3)] =
findpeaks(nonzeros(A(:,1)), 'MinPeakDistance', 10, 'MinPeakProminence',
(max(A(:,1))-min(nonzeros(A(:,1))))/4);
if isempty(PeaksArea) %if no prominent local maxima, returns the max
value
    disp('No Local AOI Maxima Detected')
    PeaksArea(1, 2) = \max(A(3:end, 1));
end
[PeaksVel(:,2),PeaksVel(:,1),w,PeaksVel(:,3)] =
findpeaks(nonzeros(A(:,2)), 'MinPeakDistance', 10, 'MinPeakProminence',
(max(A(:,2))-min(nonzeros(A(:,2))))/4);
if isempty(PeaksVel) % if no prominent local maxima, returns the max
value
    disp('No Local Vel Maxima Detected')
    PeaksVel(1,2) = max(A(3:end,2));
end
writematrix(Data, 'Data.xlsx');
save('Data.mat', 'Data');
writematrix(PeaksArea, 'PeaksArea.xlsx');
save('PeaksArea.mat', 'PeaksArea');
writematrix(PeaksVel, 'PeaksVel.xlsx');
save('PeaksVel.mat', 'PeaksVel');
%writematrix(ArrowMag, 'ArrowMag.xlsx')
save('ArrowMag.mat', 'ArrowMag');
beep
```

```
toc
```

### **Supplementary Videos**

## Video S1. Magnetic actuation of µRods in collagen

Bright-field image sequence of  $\mu$ Rods embedded in a collagen hydrogel (1 mg/mL) that were actuated by a 1.0 Hz in-plane rotating magnetic field of 55 mT magnitude. Scale bar: 100  $\mu$ m.

### Video S2. Magnetic actuation of µRods in fluorescently labeled collagen

Confocal fluorescence image sequences of  $\mu$ Rods embedded in TAMRA-labeled collagen (0.5 mg/mL). The sample was exposed to an in-plane rotating magnetic field of 73 mT magnitude, starting at 0° field orientation and going up to 180° with an increment of 10° prior to acquisition of the subsequent image. Scale bar: 50 µm.

## Video S3. Analysis of collagen network deformation

Image sequence of collagen network deformation analysis as shown in Fig. 4 a-d.  $\mu$ Rods embedded in TAMRA-labeled collagen (0.5 mg/mL) were actuated at 0.1 Hz and 73 mT in the XY plane. For analysis, collagen is shown in white, boundary of area of influence is marked in green, arrows that indicate deflection velocity are shown in blue. Scale bar: 50  $\mu$ m. For further description, consult Fig. 4a and 4d and the method description.

# **Supplementary References**

- 1 A. S. Khoo, T. M. Valentin, S. E. Leggett, D. Bhaskar, E. M. Bye, S. Benmelech, B. C. Ip and I. Y. Wong, *ACS Biomater. Sci. Eng.*, 2019, **5**, 4341–4354.
- 2 W. F. Brown and A. H. Morrish, *Phys. Rev.*, 1957, **105**, 1198–1201.
- 3 U. Chippada, N. Langrana and B. Yurke, J. Appl. Phys., 2009, 106, 63528.
- 4 D. Bradley and G. Roth, J. Graph. Tools, 2007, **12**, 13–21.