# Supplementary Materials for

## • Super-resolution traction force microscopy with enhanced tracer density enables capturing molecular scale traction

Yue Xu,[a] Chuanwen Guo,[a] Xueyi Yang,[a] Weihong Yuan,[b] Xu Zhang,[a] Yujie Sun,[b] Gang Wen,[c] Linbo Wang,[c] Hui Li, *[c] Chunyang Xiong,*[d] Chun Yang,*[a]

a *Institute of Biomechanics and Medical Engineering, School of Aerospace Engineering, Tsinghua University, Beijing 10084, People's Republic of China.*
b *State Key Laboratory of Membrane Biology, Biodynamic Optical Imaging Centre (BIOPIC), School of Life Sciences, Peking University, Beijing 100871, China.*
c *Jiangsu Key Laboratory of Medical Optics, Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou, Jiangsu 215163, China.*
d *State Key Laboratory for Turbulence and Complex System, and Department of Mechanics and Engineering Science, College of Engineering, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, People's Republic of China*
* hui.li@sibet.ac.cn.
* cyxiong@pku.edu.cn.
* yangchun@tsinghua.edu.cn.

### This PDF file includes:

Fig. S1. Regularization parameter optimization
Fig. S2. The filtering and fitting window size optimization.
Fig. S3. Quantitative estimation of traction field spatial resolution.
Fig. S4. Young's Modulus of the PAAM substrates with or without AEMA modification.
Fig. S5. Representative STED images of 40 nm beads coated on the PAAM substrates with or without AEMA modification.
Fig. S6. Representative 3D-SIM images of 40 nm or 100 nm beads coated on the PAAM substrates.
Fig. S7. Displacement field tracked by PIV or PTV.
Fig. S8. AMEA modification and high-density bead coating have no significant influence on the cell area and the cell adhesion on the PAAM substrate.
Table S1. Experimental conditions for different bead coating density.
Table S2. Recommended experimental and computational parameters for different traction measuring scales.
Table S3. A list of abbreviations and acronyms.

### Other Supplementary Materials for this manuscript include the following:

Movie S1-S2. Representative movie (Movie S1) and the zoomed view (Movie S2) of a paxillin-mCherry transfected 3t3-Swiss cell and the corresponding traction fields.
Movie S3-S4. Representative movie (Movie S3) and the zoomed view (Movie S4) of a protruding lamellipodium of an integrin α5-mCherry transfected 3t3-Swiss cell and the corresponding traction fields.
Movie S5-S6. Representative movie (Movie S5) and the zoomed view (Movie S6) of a retracting lamellipodium of an integrin α5-mCherry transfected 3t3-Swiss cell and the corresponding traction fields.

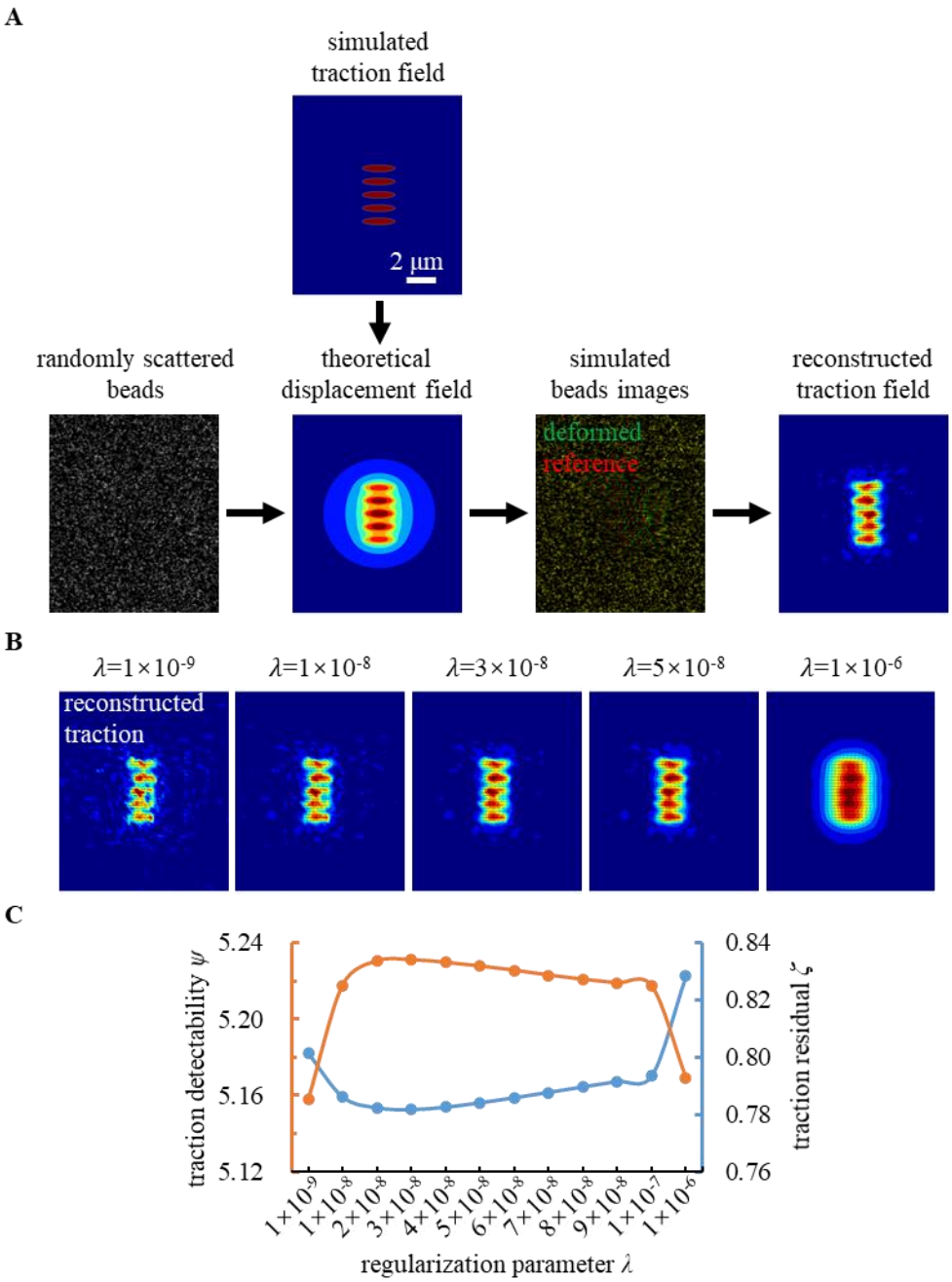


**Fig. S1** Regularization parameter optimization.
(A) Schematic of the ETD-srTFM simulation procedure. (B) Reconstructed traction field calculated by different regularization parameters. (C) Traction detectability and traction residual coefficients calculated using different regularization parameters.

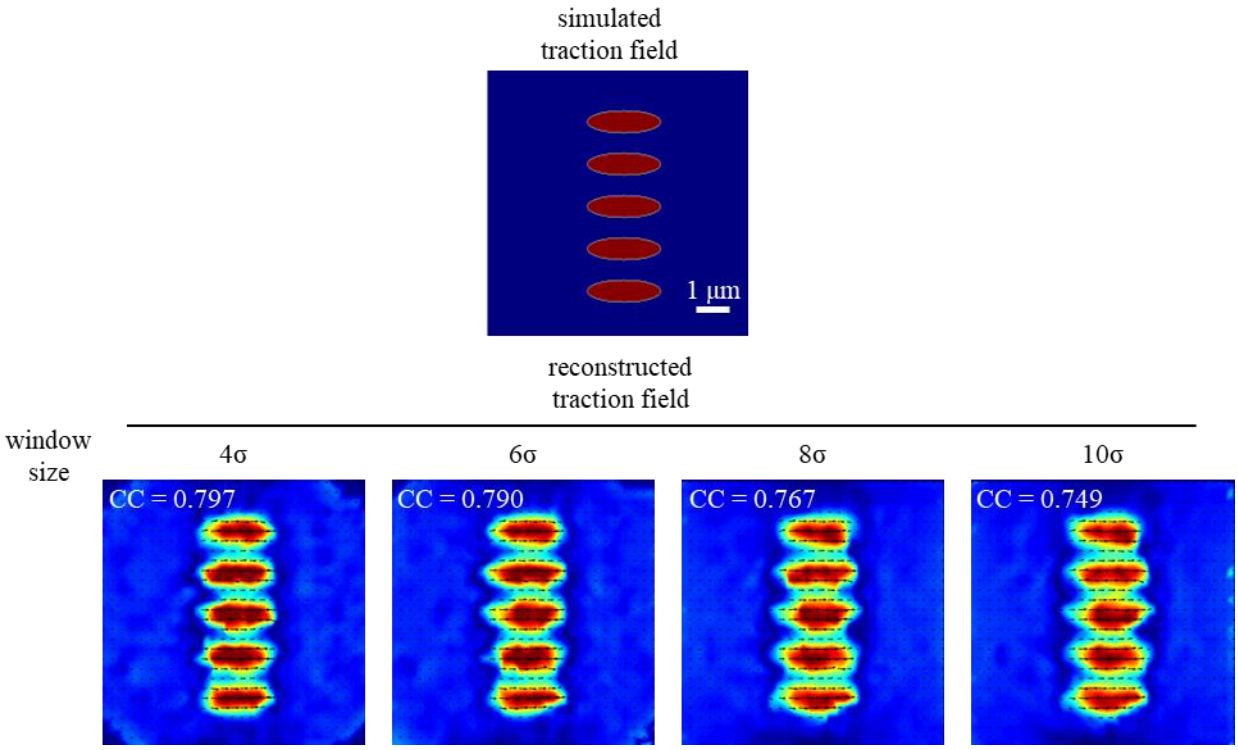


**Fig. S2** The filtering and fitting window size optimization.
We used the simulating program described in the method section 'Traction field reconstruction, simulation and regularization parameter optimization' and Fig. S1 to optimize the window size when tracking the displacement field. We input the simulated traction field showed at the top of Fig. S1 (The magnitude of the tractions was distributed spatially uniform in the red ellipses with a length of 2 μm, a width of 0.5 μm, and an interval of 1 μm. The direction of the tractions was uniformly pointed to the right side.) to the simulation program and run through step 1) to 7). We changed different filtering and fitting window size when tracking the displacement field in step 6) and the corresponding reconstructed traction fields are shown at the bottom. The normalized image correlation coefficient (CC) between the magnitude of the input and the reconstructed traction map was calculated to evaluate the accuracy of the traction reconstruction. The reconstructed traction maps show tiny difference between each other and the 4σ window size group is higher correlated with the input traction map than the others. Thus, we set the displacement tracking window size at 4σ. As the PSF standard deviation value σ is 1.44 pixels for ETD-srTFM, the window size is about 6 pixles.

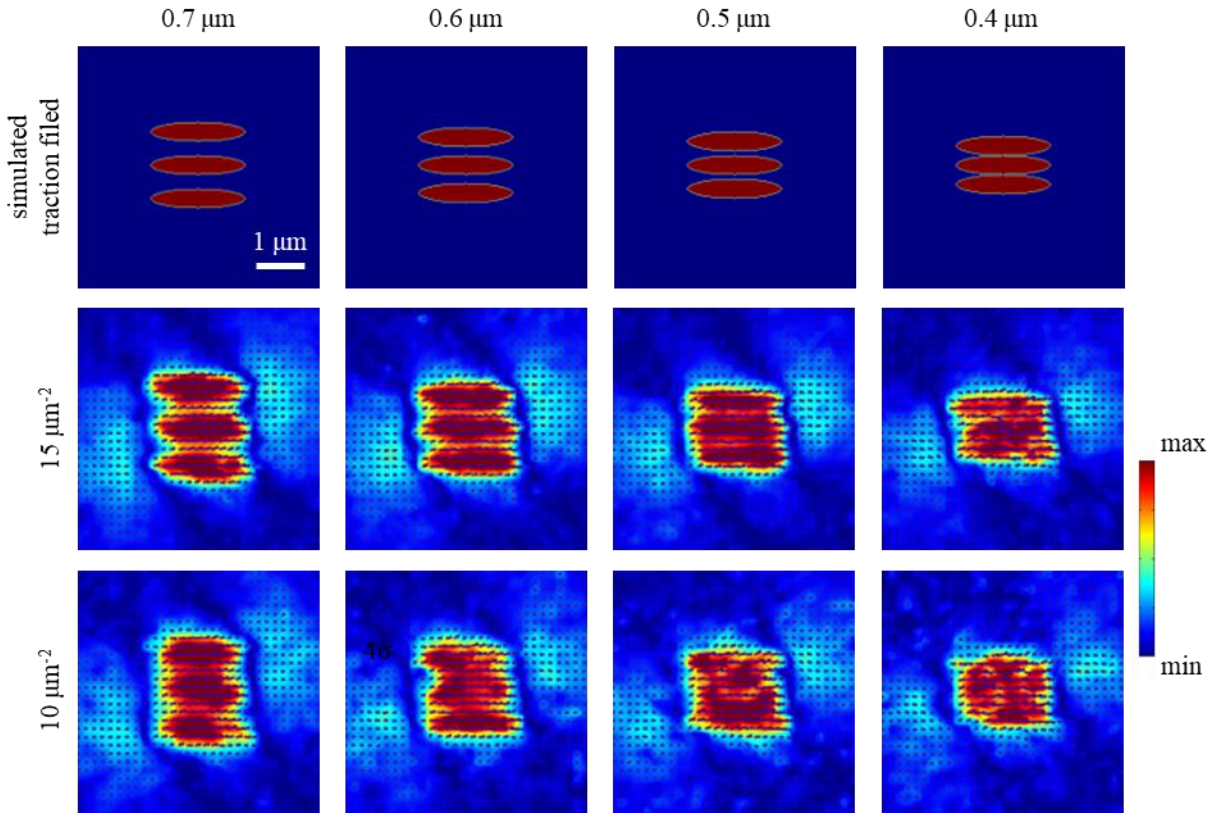


**Fig. S3** Quantitative estimation of traction field spatial resolution.
The input traction fields with the traction spot interval of 0.4 to 0.7 μm respectively (top) and the corresponding reconstructed traction fields (bottom) calculated by different simulated beads density.

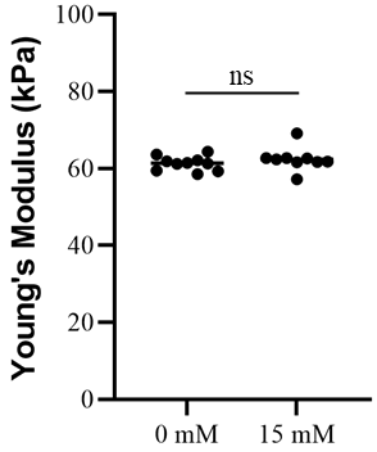


**Fig. S4** Young's Modulus of the PAAM substrates with or without AEMA modification. (ns, Student's t test, no significant difference)

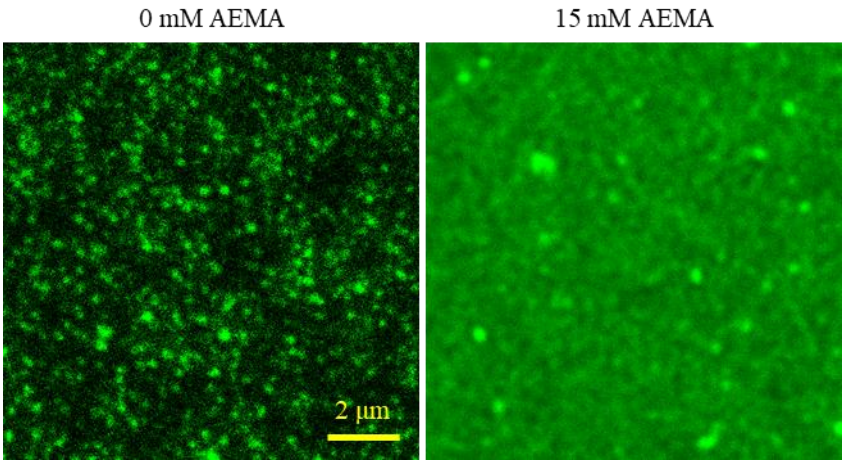


**Fig. S5** Representative STED images of 40 nm beads coated on the PAAM substrates with or without AEMA modification.

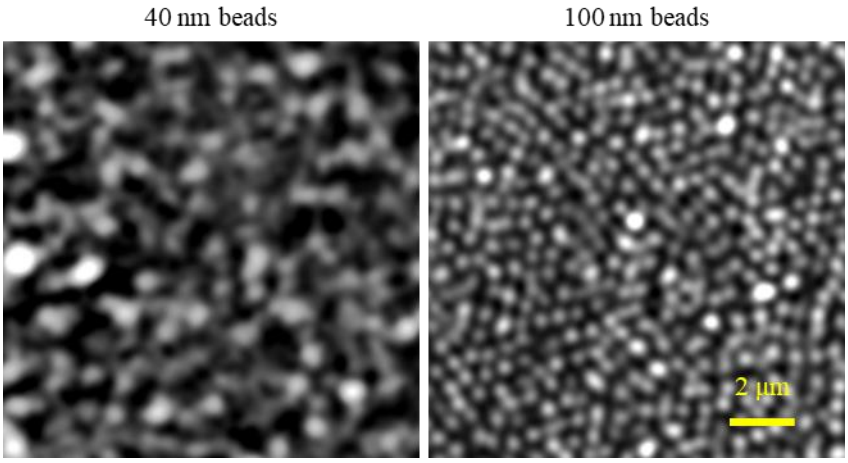


**Fig. S6** Representative 3D-SIM images of 40 nm or 100 nm beads coated on the PAAM substrates.

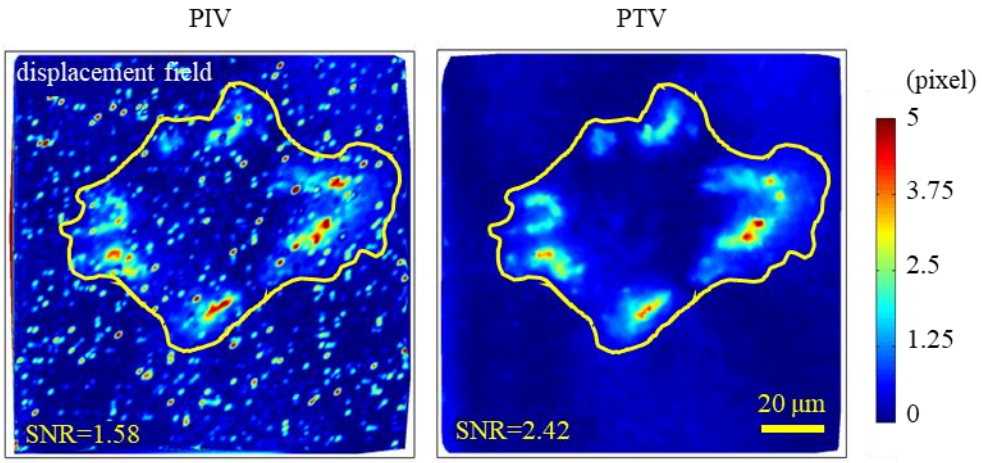


**Fig. S7** Displacement field tracked by PIV or PTV.
Yellow lines indicate the cell edge. The signal-to-noise ratio (SNR) value (at the left bottom of each map) is calculated as the ratio of the average displacement magnitude at the cell (inside the yellow circle) and that at no cell (outside the yellow circle), i.e.: $SNR = \frac{\overline{|D|_{A_{@cell}}}}{\overline{|D|_{A_{@background}}}}$, where $|D|$ is the magnitude of the displacement vectors, $A_{@cell}$ is the area inside the cell edge circle and $A_{@_{background}}$ is the area outside the cell edge circle.

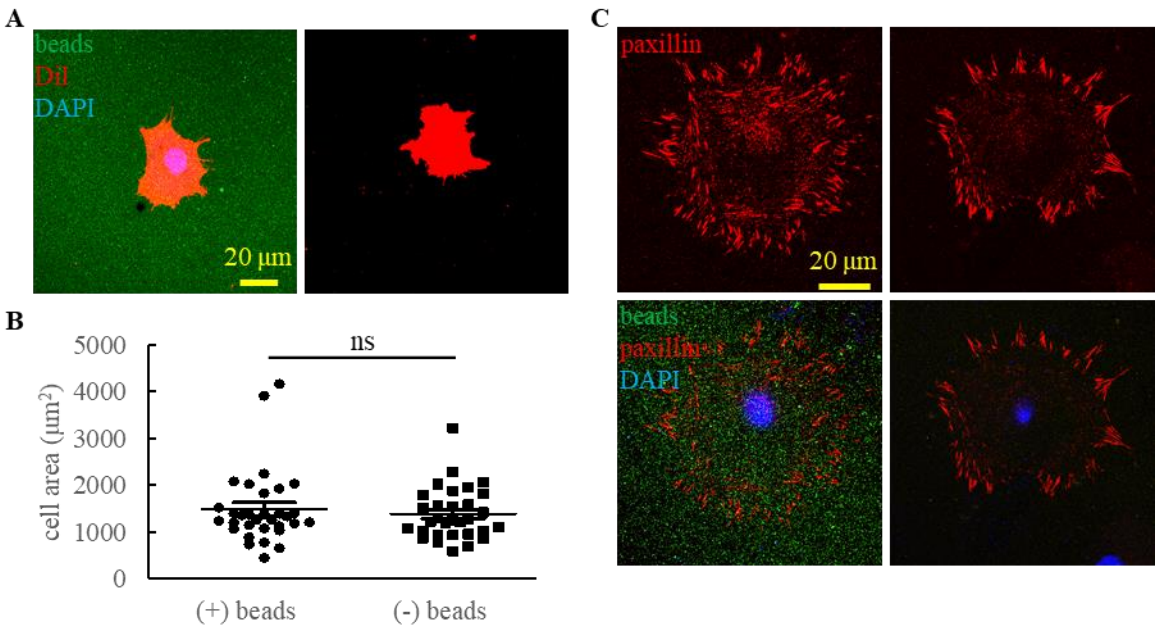


**Fig.S8** AMEA modification and high-density bead coating have no significant influence on the cell area and the cell adhesion on the PAAM substrate.
(A) Representative confocal images of DiI-stained cell membrane on the PAAM coated with or without high density beads. (B) Statistics of the cell area on the PAAM coated with or without high density beads. (ns, Student's t test, no significant difference) (C) Representative confocal images of immunofluorescent staining of FA marker protein, paxillin, on the PAAM coated with or without high density beads.

| sample number | AEMA concentration (mM) | 100 nm beads dilution rate | sedimentation time | bead sampling density ($\mu m^{-2}$) |
|---|---|---|---|---|
| 1 | 2.5 | 1：5000 | 30 min | 0.58±0.01 |
| 2 | 2.5 | 1：1000 | 30 min | 1.25±0.08 |
| 3 | 0 | 1：1000 | 2 h | 1.77±0.08 |
| 4 | 2.5 | 1：5000 | O/N | 2.26±0.08 |
| 5 | 7.5 | 1：1000 | 30 min | 3.92±0.11 |
| 6 | 15 | 1：1000 | 2 h | 4.95±0.28 |
| 7 | 0 | 1：1000 | O/N | 5.11±0.55 |
| 8 | 15 | 1：1000 | 4 h | 12.53±1.02 |
| 9 | 15 | 1：1000 | O/N | 15.03±0.89 |

**Table S1** Experimental conditions for different bead coating density. (O/N, overnight)

| | large scale TFM | confocal-TFM | ETD-srTFM |
|---|---|---|---|
| microscope | wide field fluorescent microscope / confocal | confocal | 3D-SIM |
| lens (magnification / NA) | 40× NA 0.75 | 100× NA 1.40 | 100× NA 1.49 |
| pixel size (nm) | 320 | 120 | 30 |
| bead diameter (nm) | 200 | 200 | 100 |
| PSF standard deviation σ (pixels) | 0.54 | 0.77 | 1.44 |
| bead sampling density ($\mu m^{-2}$) | ~ 0.5 | ~ 2 | ~ 15 |
| regularization parameter ($\lambda$) | $1\times10^{-5}$ | $6\times10^{-8}$ | $3\times10^{-8}$ |
| traction measuring scale (μm) | > 10 | 3 - 10 | < 3 |

**Table S2** Recommended experimental and computational parameters for different traction measuring scales.

| Abbreviations & Acronyms | |
|---|---|
| AEMA | aminoethyl methacrylate hydrochloride |
| AFM | atomic force microscope |
| DAPI | 4′,6-Diamidino-2-phenylindole dihydrochloride |
| DiI | 1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate |
| ECM | extracellular matrix |
| EDC | 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride |
| EGFP | enhanced green fluorescent protein |
| ETD-srTFM | enhanced tracer density super-resolution microscopy |
| FA | focal adhesion |
| FWHM | full width at half maximum |
| LC-SLM | liquid crystal spatial light modulator |
| MES | 2-(N-Morpholino)ethanesulfonic acid |
| MTFM | molecular tension fluorescence microscopy |
| NA | numerical aperture |
| PAAM | polyacrylamide |
| PBS | Phosphate buffered saline |
| PIV | particle image velocimetry |
| PSF | point spread function |
| PTV | particle tracking velocimetry |
| FTTC | Fourier–Transform Traction Cytometry |
| SIM | structured illumination microscopy |
| STED | stimulated emission depletion |
| sulfo-NHS | N-Hydroxysulfosuccinimide sodium salt |
| sulfo-SANPAH | sulfosuccinimidyl 6-(4′-azido-2′-nitrophenylamino)hexanoate |
| TEMED | N,N,N',N'-Tetramethyl ethylenediamine |
| TFM | traction force microscopy |
| TIRF | total internal reflection fluorescence |

**Table S3** A list of abbreviations and acronyms.