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Supplemental Information

Measurement of Skeletal Muscle Fiber Contractility with High-Speed Traction Microscopy

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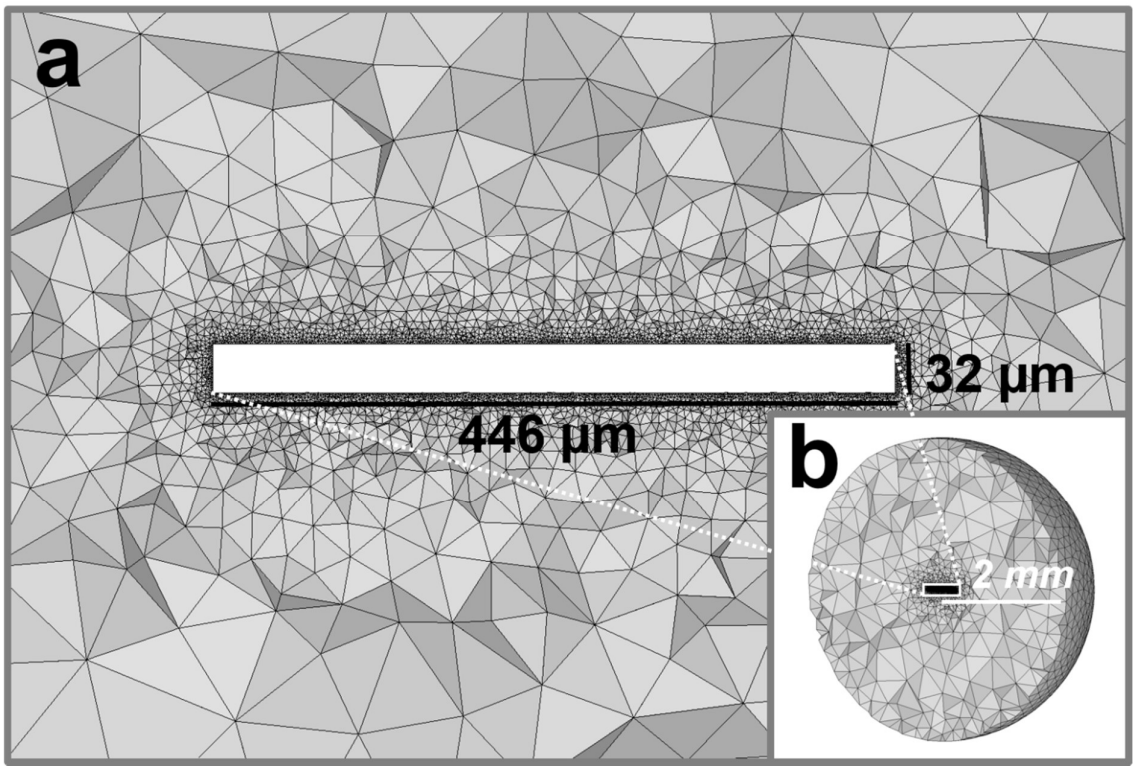
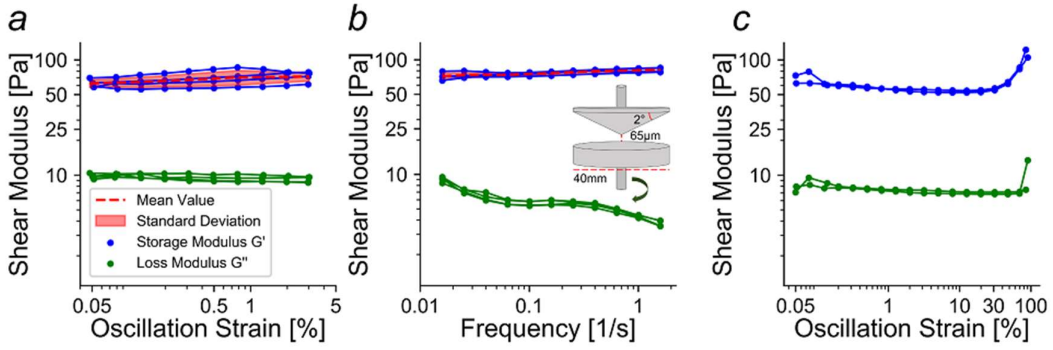
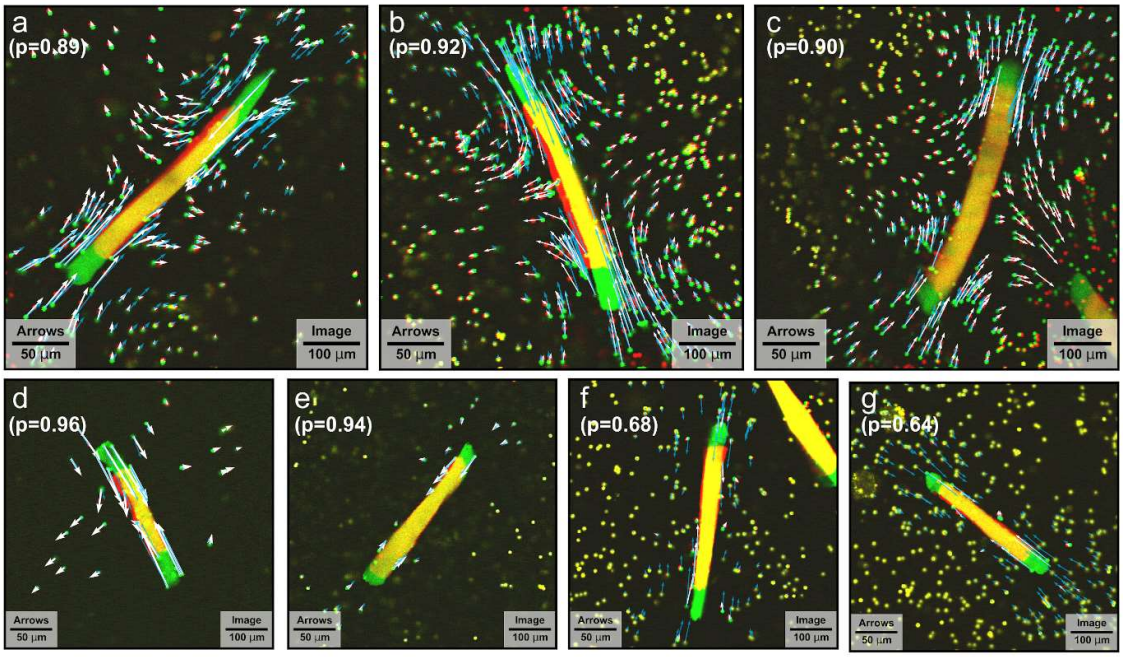


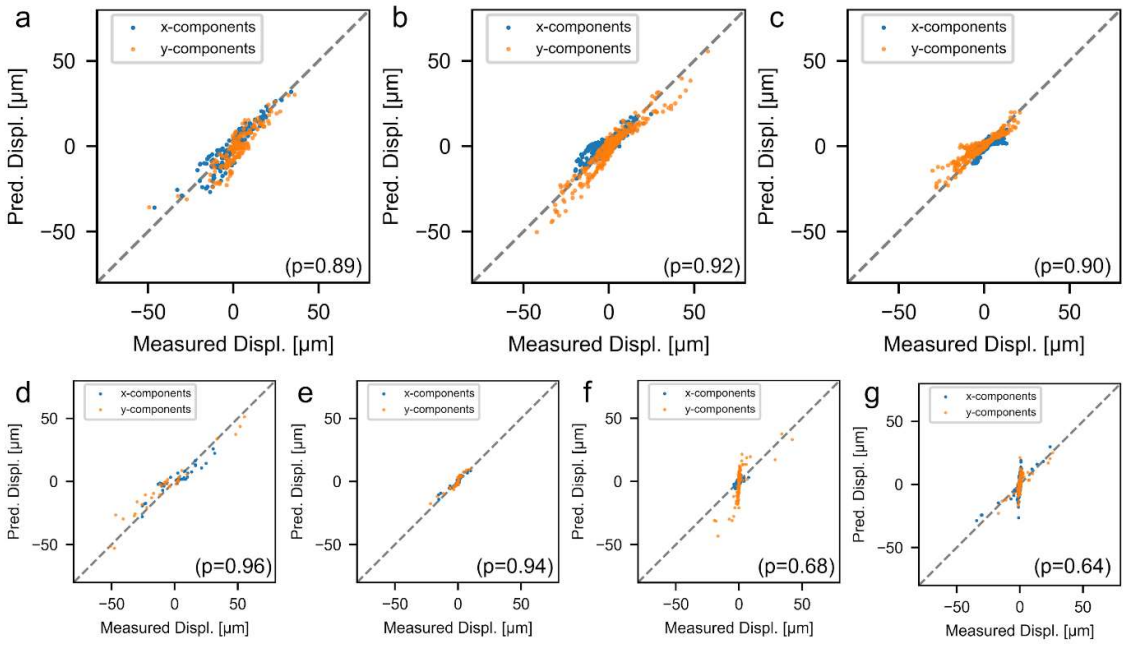
Supplementary Fig. 1: Tetrahedral mesh used in the finite element simulations: **a:** The cell is modelled as a cylindrical inclusion with a length of 446 µm and a diameter of 32 µm. **b:** The geometry of the experimental setup is modelled as a Matrigel sphere with a radius of 2 mm with the cell at its center.



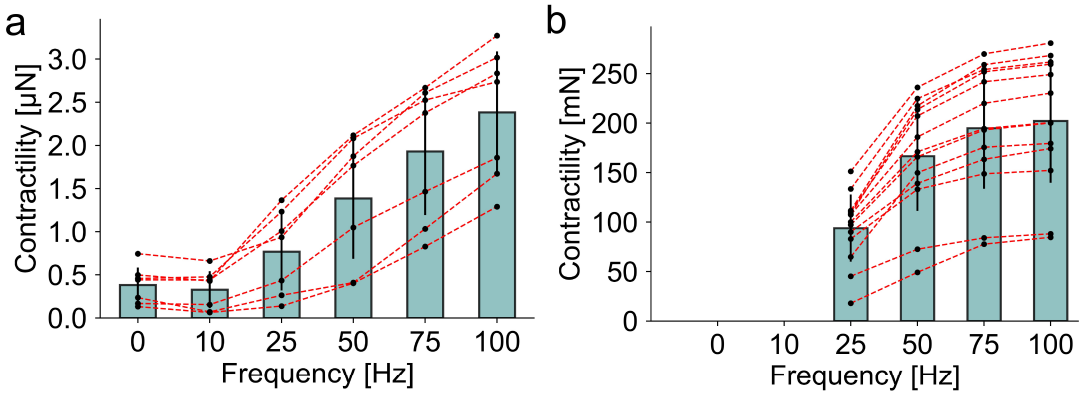
Supplementary Fig. 2: Cone-plate shear-rheometer measurements of 10 mg/ml Matrigel hydrogels. **a:** Shear modulus for various strains (0.05 % - 3 %) at a fixed frequency of 0.1 rad/s, measured in four independent experiments. **b:** Shear modulus for a range of frequencies (from 0.1 - 10 rad/s) at a fixed strain of 0.5%, measured in three independent experiments. **c:** Shear modulus at high strains (0.05% - 100%), measured in two independent experiments. We observe predominantly linear elastic properties in all measurements.



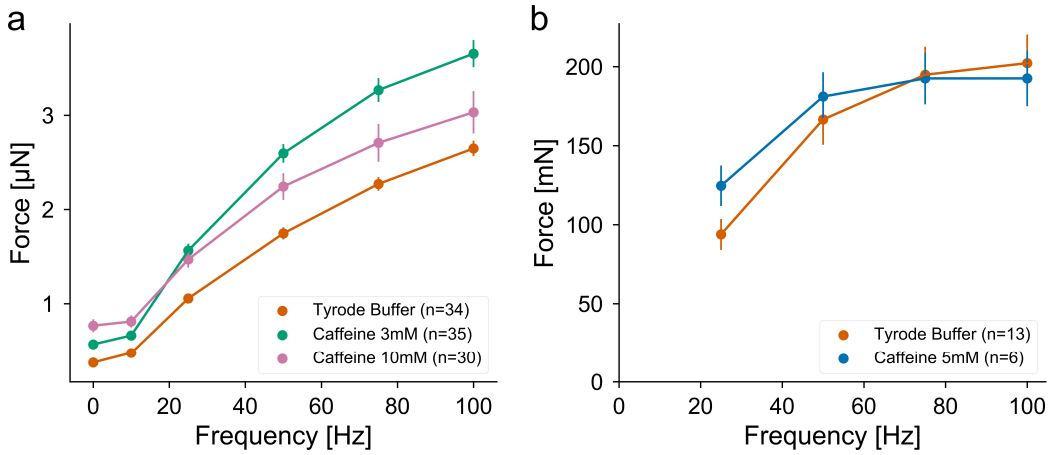
Supplementary Fig. 3: Matrix and fiber deformations in the mid-plane of FDB muscle fibers. Images taken in the relaxed state (green) are superimposed with images taken the contracted state (red). FDB fibers are electrically stimulated with 1 ms long pulses every 10 ms (100 Hz) for a duration of 300 ms. White arrows show the measured deformations, blue arrows show the predicted deformations from FE modelling. The Pearson correlation coefficient (p) between predicted and measured deformations is indicated in the left upper corner of each image and is on average 0.85 ± 0.12 (mean ± sd). Fibers in a-c show good agreement between predicted and measured deformations. In d) and e), the bead density is low, but the measured deformations are in good agreement with FE simulations. In f) and g) we find substantial deviations between predicted and measured matrix deformations. Small or absent bead movements indicate poor attachment between the FDB fibers and the surrounding matrix.



Supplementary Fig. 4: Predicted matrix deformations vs. measured matrix deformations in x-direction (blue) and y-direction (orange), for all FDB fibers shown in Fig. S3. Line of identity is shown in grey. Each point indicates the displacements from one fiducial marker bead. The Pearson correlation coefficient (p) between predicted and measured bead displacements is indicated in the right lower corner of each graph.



Supplementary Fig. 5: Force-Frequency response of single flexor digitorum brevis (FDB) muscle fibers (**a**) and extensor digitorum longus (EDL) whole muscle preparations (**b**). A similar frequency-dependent increase in contractility is observed in both cases.



Supplementary Fig. 6: Force versus stimulation frequency relationship for single flexor digitorum brevis (FDB) muscle fibers embedded in Matrigel (**a**) and extensor digitorum longus (EDL) whole muscle preparations (**b**) treated with different concentrations of caffeine dissolved in Tyrode buffer. In both preparations, a dose-dependent increase in force is observed. At high stimulation frequencies, the contraction-enhancing effect of caffeine vanishes in whole muscle preparations, whereas in single cells, the effect of caffeine persists. Data points represent mean ± se of *n* samples as indicated in the legend.

Supplementary Video 1: Maximum contractions in response to different stimulation frequencies of a single flexor digitorum brevis (FDB) fiber stained with Mag-Fluo-4 Ca^{2+} indicator and embedded in Matrigel. Images are recorded with a confocal microscope. Fluorescent beads are used as fiducial markers for quantifying matrix deformations around the cell.

Supplementary Video 2: Contractions of several FDB muscle fibers (typically smaller than 500 µm) together with interosseus muscle fibers (typically larger than 700 µm) embedded in Matrigel for different stimulation frequencies recorded with a confocal microscope. The left panel shows the bright-field channel; the right panel shows the fluorescent channel (Mag-Fluo-4 Ca^{2+} indicator). Videos show data acquisition in real-time (30 frames/s).