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Cellular mechanosensing on cell-scale stiffness gradient substrate†

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1 Supplementary Figures

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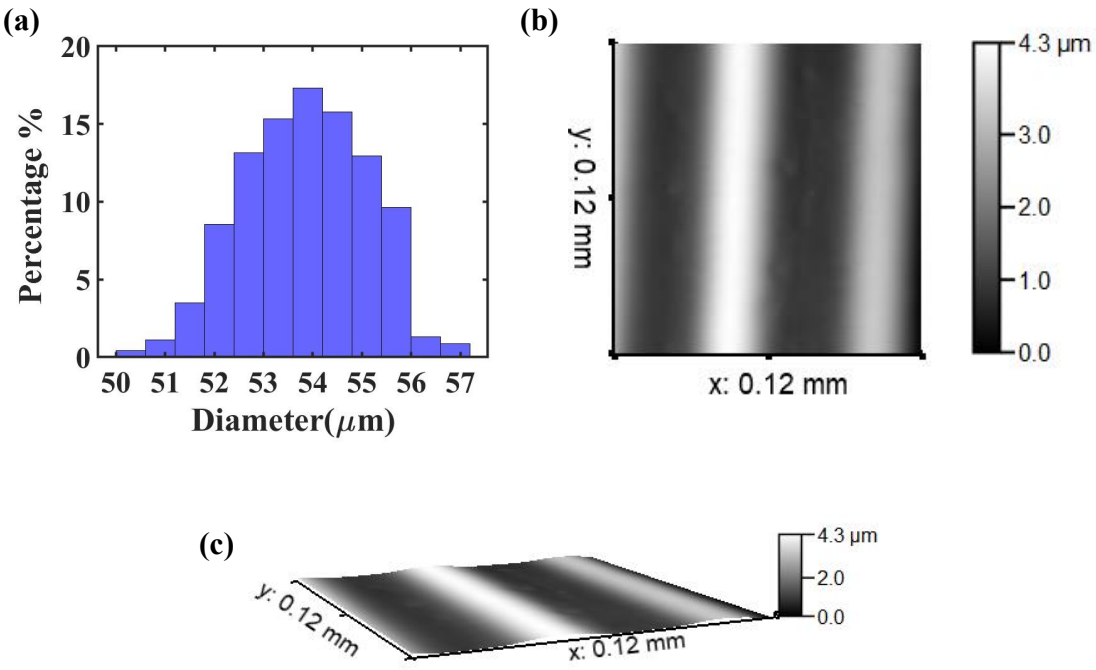

Fig. 1 a) Analysis of rods diameter consistency across Multiple locations.b)Surface topography map of the substrate captured through atomic Force Microscopy (S-1). c) 3D view of the substrates' surface hight profile(S-1).

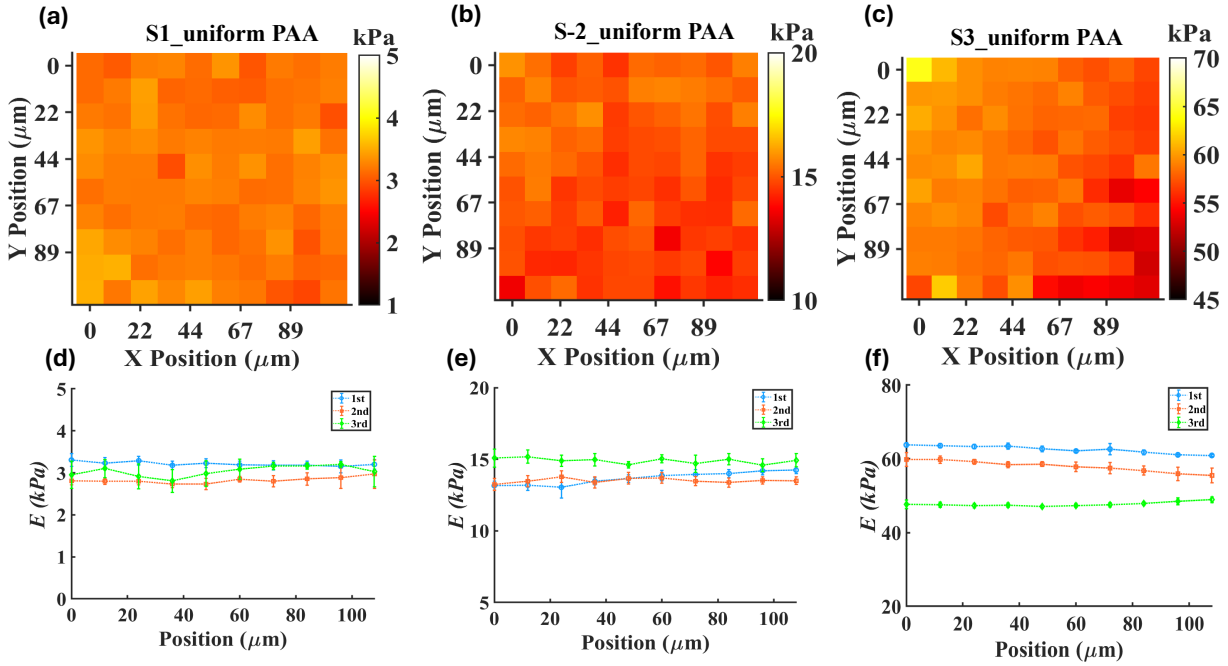

Fig. 2 The panels labeled with a) to c) Shows the stiffness map of the PAA gels used to prepare the substrates S-1, S-2, and S-3, respectively. The stiffness map covers a scan area of 108 μm × 108 μm across a 10×10 grid (n=3 for each composition). d), e) and f) presents the average bulk stiffness of the different compositions PAA gels (over an area of 108 μm × 108 μm across a 10×10 grid)(n=3 for each composition).

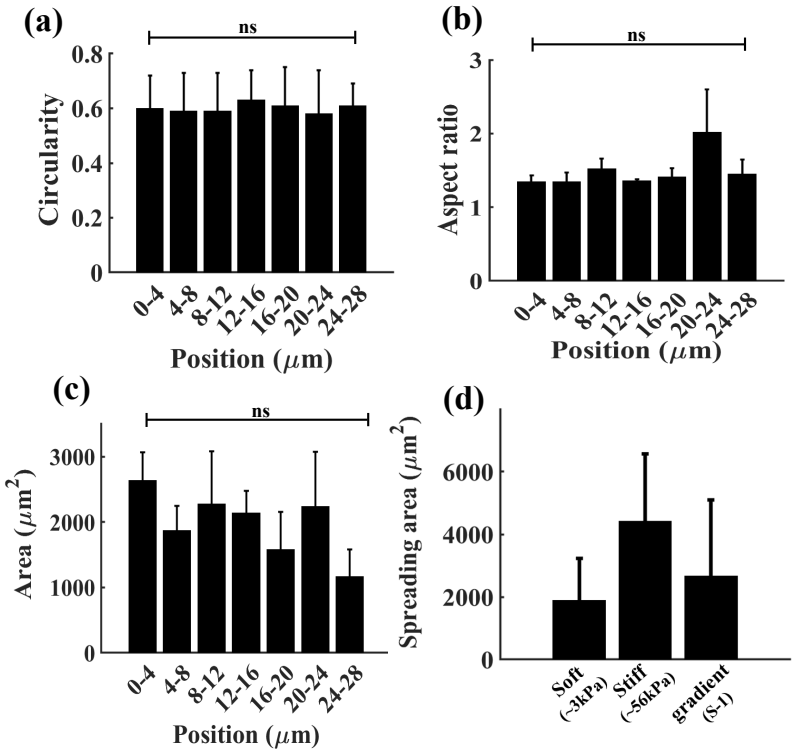

Fig. 3 a) and b) Shows the circularity and Aspect ratio of the cells' nuclei at different position(S-1), with a bin size ± 4 μm from the peak of the rods taking as reference point, respectively. c) Represents the cells spreading area at different position, taking the peak of the rods(where the thickness of the gel was minimum) as a reference point on S-1 ($N > 100$ over n=3 replicates) . D) The cell spreading area on the soft($\sim$ 3kPa)($N > 60$ Cells), stiff($\sim$ 56 kPa)($N > 50$ cells) and gradient substrates S-1($N > 80$ Cells), respectively(data represents as mean $\pm$ SD). ns: $p > 0.05$., not significant.

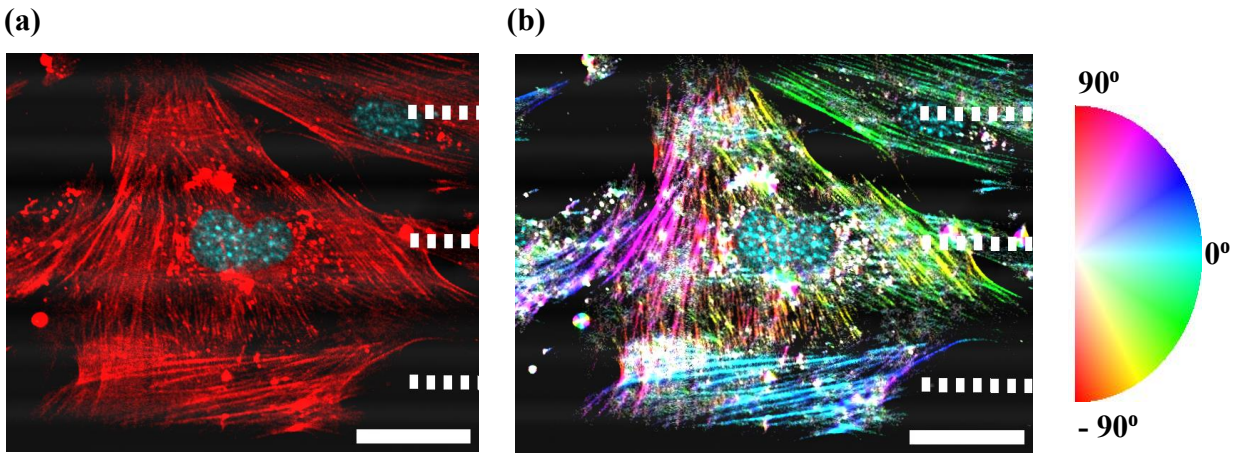

Fig. 4 a) Fluorescence image illustrates the cytoskeletal organization of actin stress fibers in 3T3 fibroblast cells seeded on an S-2 substrate. b) Colors represent locally orders actin domains. The color wheel indicates the orientation angles of the actin stress fibers, with each color corresponding to a specific angle. Dashed lines indicating the peak of the rods, which corresponds to highest stiffness region. scale bar= 50 μm

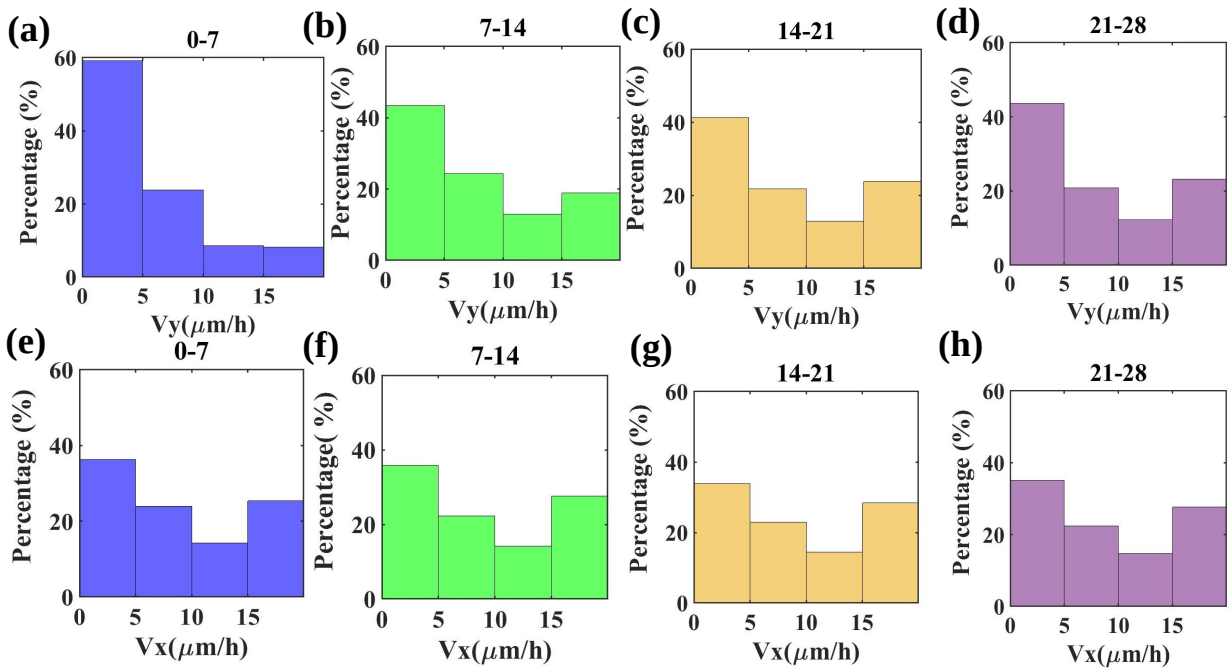

Fig. 5 The panels labeled with a) to d) and e) to h) show the distribution of cells' vertical speed values and horizontal speed values over relative position of different location from the peak of the rods of the substrate, taking the peak of the rods(where the thickness of the gel was minimum) as a reference point, respectively on S-1($N > 80$ over n=4 replicates). Numbers on top of the images representing the respective region of the distribution(μm).