Electronic Supplementary Material (ESI) for RSC Advances.

Cell culture

NIH 3T3 fibroblasts were grown in Dulbecco's Modified Eagle Medium supplemented with 10% Fetal Bovine Serum (both Life Technologies, Carlsbad, CA, USA), 2 mM L-glutamine (Sigma, St. Louis, MO), and 100 U/ml penicillin 100 µg/ml streptomycin at 37 °C at 5% CO2. In order to maintain the same conditions during the experiments, 4x104 cells were counted and put in a 35 mm Willco-dish (Willcowells BV, Amsterdam, The Netherlands) in a temperature and humidity controlled environment (using a micro-incubator by Bioscience Tools, San Diego, CA, USA).

In order to avoid cell adhesion onto plate surface, previously, 35 mm Willco-dishes were coated treating the surface in a low pressure O2 plasma system (Femto System, Diener Electronic GmbH & Co. KG, Ebhausen, Germany) for micro-cleaning and to activate their surfaces; they were then spin-coated with 50 µl of Fluorolink PFPE S10 (Solvay Polymers Ltd, Warrington, UK) at 10000 rpm for 2 min and incubated under vacuum for 30 min. After incubation, Fluorolink was de-activated adding de-ionized water and Petri dishes were rinsed with Ethanol 100% (Delchimica Scientific Glassware, Naples, Italy) in order to discard Fluorolink in excess, then they were sterilized with a UV lamp treatment for 30 min.

Subsequently, 20 mM HEPES solution (Sigma-Aldrich, St. Louis, MO, USA) and RGD functionalized beads were added to the medium and a cover glass was positioned to spread out the drop of cells and beads.

RGD conjugation to polystyrene microbeads

The polystyrene microspheres (PS) carboxylate-modified 4% solids, diameter 8 µm, were purchased from Thermo Scientific. Arginine–Glycine–Aspartic acid (RGD, FW=346.3 $gmol^{-1}$), *N*-Hydroxysuccinimide 97% (NHS) and *N*-(3-dimethylaminopropyl)-*N*`-ethylcarbodiimide hydrochloride bio extra (EDAC) were

purchased from Sigma-Aldrich. Triethylamine (TEA) and water super purity solvent were purchased from Romil Pure Chemistry. Reagent and solvent were used without further purification unless otherwise specified. Spectra/Por® Dialysis membrane MWCO: 6-8000 was purchased from Spectrum Laboratories, Inc. 1.8 mg of NHS and 4.6 mg of the EDAC excess (0.016 mmol and 0.024 mmol) was added to 1.5 mL of aqueous solution beads (d_{PS}=1.05 mg/cm^3; 63 mg of beads). 3.4 µL of TEA excess (0.023 mmol) was added to 6.76 mg of RGD (0.016 mmol) dissolved in 500 µL of Romil-SpS water. The two solutions were mixed and left at ambient temperature under vigorous stirring for 4 h.

The beads were dialysed using Spectra/por® dialysis membrane MWCO: 6-8000 and Romil-SpS water for 48 h. Next, the microspheres were precipitated in dialysis with phosphate buffer pH=7.4 for 12 h, centrifuged at 1500 rpm and stored at 4 °C. The bioconjugation ratio is equal to 0.107 (bioconjugation degree is about 0.1 per 1 mg of microparticles). FTIR experiments were performed by using NICOLET 6700 Thermo Scientific and considered the spectra in the 2000-1000 cm^{-1} region of polystyrene microspheres carboxylate-modified and polystyrene microspheres RGD conjugated. Microspheres polystyrene and microspheres RGD conjugated were dialysed into Romil-SpS water for 48 h utilising Spectra/por® dialysis membrane MWCO: 6-8000, successively centrifuged at 1700 rpm, dried under vacuum and included in KBr salt. Beads spectra presented the characteristic bands of polystyrene at 1496 cm^{-1}, a peak at 1683 cm^{-1}, attributed to carboxylic groups, and a further peack at 1548 cm^{-1} relative to the amide groups (amide II), confirming the conjugations with the RGD peptides.

Gene Transfection

Lipofectamine LTX reagent (by Life Technologies, lot 1468812) was used to transfect the pCMVLifeAct-TagRFP (ibidi) mammalian expression vector in NIH/3T3 cells in order to visualize filamentous actin (F-actin) in living cells. After 15 min of incubation with 0.75 μg of pDNA in lipoplexes, cells were returned to culture with complete medium and grown at 37 °C and 5% CO2.

Set-Up of HOT, DH and fluorescent moduli

Concerning the HOT modulus, the infrared (IR) trapping laser emitted at 1064 nm, the beam was expanded to fit the aperture of a Spatial Light Modulator or SLM (Holoeye - PLUTO-NIR phase only) operating in reflection mode. Then, the laser beam was injected into a Microscope Objective MO (100x N.A.=1.2) through a standard 4f optical configuration. The sample chamber was positioned beyond the MO. By HOT, we trapped and manipulated micrometer latex beads in order to anchor them to suspended cells. Doing so, the optical forces were exerted on the micro-spheres, thus avoiding the direct interaction of the laser beam with cells. Specifically, by functionalizing the microbeads with RGD peptides, we gave the cell n points of adhesion and stimulation (where n is the number of HOT trapped microbeads). Then the cell had the chance to assemble its cytoskeleton and react to mechanical stimuli applied by HOT. For our purposes we designed trapping sites able to trap polystyrene beads 8 μm-sized. A fiber-coupled, solid state laser emitting at 532 nm was used to build the Mach-Zehnder interferometer, allowing recording a sequence of digital holograms of the sample during the experiment (light green path in Figure 1(a)). From these sequences we reconstructed the Quantitative Phase Maps (QPMs) of the samples. The third part of the setup is the fluorescence modulus. Light from a fluorescence lamp (X-cite series 120pc Lumen Dynamics) was directed on the sample with a combination of excitation and emission (Tritc) filters, suited for the

fluorophore used in the experiment. All images were recorded with an USB U-Eye Camera (from IDS), 1280x1024 pixels (pixel size 5.3 μm), recording at 25fps.

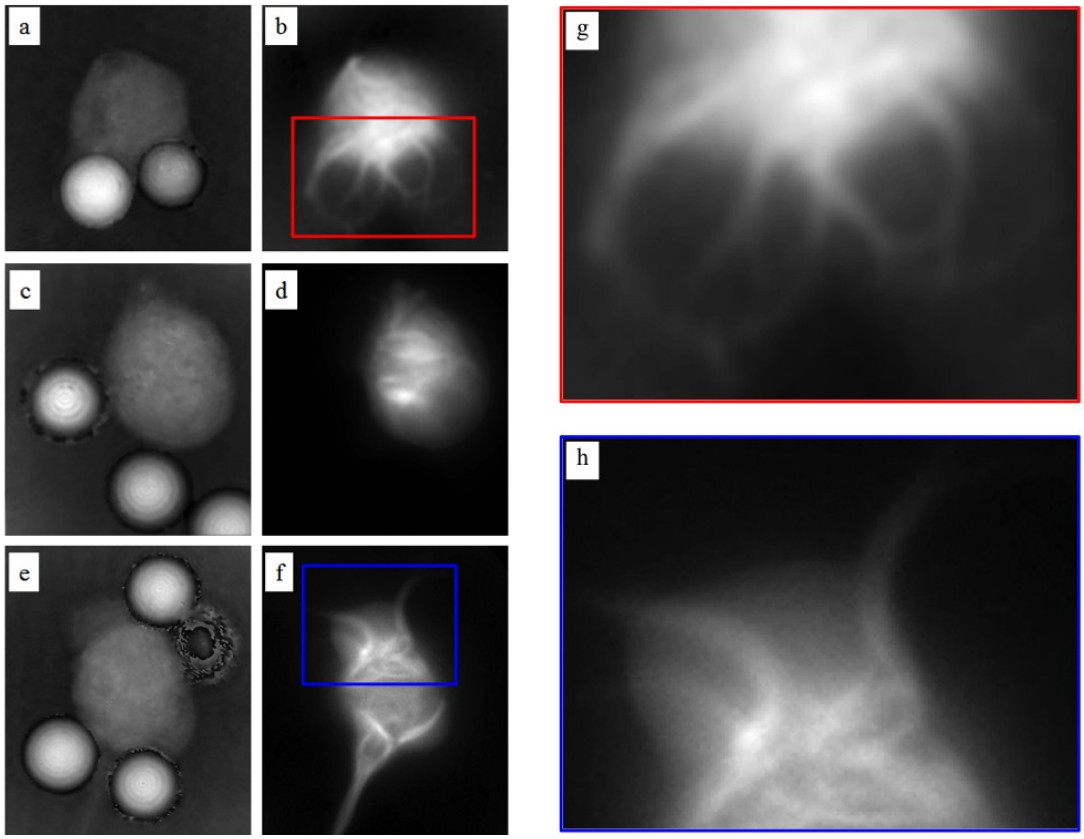

Figure S1 (a,c,e) QPMs and (b,d,f) fluorescence images of different trap configurations. (a-d) Two beads and (e,f) four beads are attached to the fibroblast, in suspension. The cell feels as only foothold the beads, reorganizing itself consequently. Zoom in images (g,h) of (b,h) configurations shows actin accumulation and filaments on microsphere surfaces indicating cell anchor points (focal adhesions).

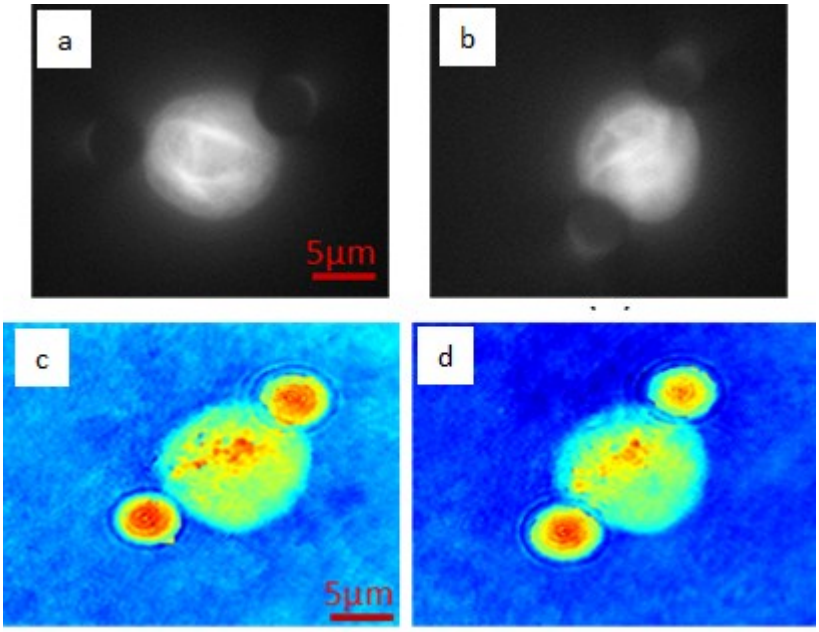

Figure S2 (a) and (b) are two fluorescent images of a LifeAct-RFP transfected cell clamped between two microspheres; the images are recorded with a time interval of 15 min and clearly show the presence of actin aggregates. After recording the image, (a) the system bead-cell-bead is rotated by HOT modulus in order to prove the non-adhesion of the cell onto the petri dish; (c) and (d) are two QPMs of the cell retrieved by DH showing an enhancement of the OPD signal in correspondence of the line connecting the two microspheres.

Table S1. Elastic constant of optical trap measured at different time points of the experiment. For each measurement, we calculate an error equal to 0.16 pN/ μ m.

Table S1 – Trap Elastic Constant K [pN/μm]

	Left Fixed Bead		**Right Moving Bead**	
	x axis	*y* axis	*x* axis	*y* axis
Before approach[1]	2.7	2.1	2.6	2.1
After approach	2.7	2.4	2.6	2.8
After stretching	8.1	1.9	29.4	3.3

[1] Before approach, x and y elastic constants should be ideally equal, but we report the measured average values of elastic constants with standard deviations of $\sigma = 0.16$ pN/μm, thus we are almost within the statistical error (3σ). Moreover, it is possible that our trap spots present some unperceivable distortion (i.e. astigmatism), so that they do not have exactly the same intensity in the two directions (x and y).