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Electronic Supplementary Information (ESI)

Sample Preparation

Synthesis of surface RGD functionalized beads

At 1.5 mL of aqueous solution of carboxyl-modified 4% solids polystyrene microspheres 8 µm (Thermo Scientific, Indianapolis, IN, USA) (dPS=1.05 mg/cm3; 63 mg of beads) was added 1.8 mg of N-Hydroxysuccinimide 97% (NHS, Sigma-Aldrich, St. Louis, MO, USA) and 4.6 mg of N-(3-dimethylaminopropyl)-N`-ethylcarbodiimide hydrochloride bio extra (EDAC, Sigma-Aldrich, St. Louis, MO, USA) excess (0.016 mmol and 0.024 mmol). At 0.016 mmol of tripeptide Arginine–Glycine–Aspartic acid (RGD, FW=346.3 gmol-1, Sigma-Aldrich, St. Louis, MO, USA), dissolved in 500 µL of water super purity solvent (Romil Pure Chemistry, Waterbeach, UK) was added 0.023 mmol of Triethylamine (TEA, Romil Pure Chemistry, Waterbeach, UK) excess (0.023 mmol). The two solutions were mixed and leaved at room temperature under vigorous stirring for 4 hours. Beads were then dialyzed into water super purity solvent for 48 hours utilizing Spectra/Por® dialysis membrane MWCO:.6-8000 (Spectrum Laboratories, Inc, Rancho Dominguez, CA, USA), and precipitated in dialysis with phosphate buffer pH=7.4 for 12 hours, centrifuged at 1200 rpm and finally stored at +4°C.

FTIR characterization

FTIR experiments were performed by using NICOLET 6700 (Thermo Fisher Scientific, Waltham, MI, USA) and considered the spectra in the 2000-1000 cm^{-1} region of carboxylate-modified polystyrene microspheres and RGD conjugated polystyrene microspheres. RGD conjugated microspheres were dialysed into water super purity solvent for 48 hours utilising Spectra/Por® dialysis membrane MWCO:.6-8000, successively centrifuged at 1700 rpm, dried under vacuum and included in KBr salt. Beads showed at 1452 cm^{-1} and 1496 cm^{-1} characteristics bands of polystyrene and 1683 cm^{-1} pick attributed at carboxylic groups and also spectra at 1653 cm^{-1} (amide I) and 1548 cm^{-1} (amide II) picks attributed to the amide groups, indicating to the RGD conjugation.

Cell culture

NIH-3T3 cells were cultured in Dulbecco's Modified Eagle Medium + 10% Fetal Bovine Serum (both Life Technologies, Carlsbad, CA, USA) and incubated at 37°C and 5% CO_2. In order to maintain the same conditions during the experiments, cell were put in a 35mm Willco-dish (Willcowells BV, Amsterdam, The Netherlands), in a temperature and humidity controlled environment, using a TC-MIS-35 micro-heater (Bioscience Tools, San Diego, CA, USA) adding 20 mM HEPES solution (Sigma-Aldrich, St. Louis, MO, USA) to the medium.

Tracking Method by Lateral Shear to detect Brownian motion

The setup depicted in Fig.1a is a standard 4f configuration used to realize an HOT arrangement. Computer Generated Holograms (CGH) are calculated by Iterative Fourier Transform Algorithm (IFTA) and displayed by the SLM to trap particle in designed positions in the sample volume. For our purposes we design two trapping sites able to trap two polystyrene beads. By refreshing the CGH we manage the 3D position of each trapping sites independently. Once the microbeads are trapped, we perform the calculation of their positions by the new method based on lateral shear.

In general, video tracking techniques open the possibility to track multiples probes conversely from BFP method that uses Quadrant Photodiode. DH potentiality as video tracking method has been previously proved, but here we demonstrate that a fast and accurate particle positioning can be obtained exploiting the intrinsic Brownian motion by a single step numerical analysis. Although, we cannot compare directly the proposed strategy with the BFP method, we compare the method with standard tracking strategy based on centre of mass calculation.

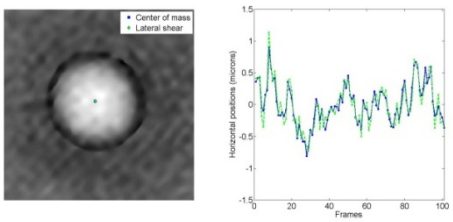

Fig. 8 comparison between LSI based method and centre of mass based method.

As show in Fig.8b, we observe a great closeness between the estimated position with the two compared method. Typically, sample detection is achieved applying a threshold filter on the QPM numerically reconstructed at the nominal distance (i.e. the reconstruction distance roughly measured during the recording step). This procedure is mainly devoted to label micro-objects and define the ROI in which the 3D coordinates will be computed. The focal plane estimation is obtained by refocusing algorithms, based on the cumulated edge detection, that quantify the image sharpness of the amplitude reconstructions.

Finally, we measure the correlation coefficient between the displacement of the two trapped beads revealing an enhancement of the correlation in the case of beads-cell system. In fact, the correlation coefficient between the estimated horizontal displacements of trapped beads without cell is equal to 0.032, instead in case of beads-cell system the correlation on the horizontal displacements is equal to 0.532. If we compare the vertical movements, the correlation for only-trapped beads is 0.004 and 0.069 for the beads-cell system.

Comparison between MBF Method ant Lateral Shear Method for tracking

The transverse coordinates calculation by MBF is achieved finding the centroid of the binary filter obtained from two subsequent phase reconstruction of the cell under analysis. So the process is divided into the following steps, given the 3D position of the considered cell and its unwrapped phase map at time *k*:

1. Reconstruction of the subsequent hologram (*k*+1) at in-focus distance given by the refocusing with the Tamura coefficient minimization;
2. Wrapped phase reconstruction and unwrapping;
3. Computation of the thresholding filters of both unwrapped phase maps;
4. Calculation of MBF;
5. Estimation of transverse coordinates for the (*k*+1)th frame.

The method proposed here and based on lateral shear does not require the calculation of the unwrapped phase maps, therefore, the two techniques have in common only the step 1. In fact, given both complex reconstructions of kth and (k+1)th frames, the Lateral shear-based method provides the transverse coordinates by a simple 2D fitting, as shown in Fig.2 (b).