Optical tracking of microobjects within living cells

Jan Ježek[a], M. Vařecha[b], J. Amrichová[b], M. Kozubek[b], T. Čižmár[a], P. Zemánek[a]

[a]Institute of Scientific Instruments of Academy of Sciences of Czech republic, Královopolská 147, 612 64 Brno, Czech republic
[b]Faculty of Informatics, Masaryk University Brno, Botanická 68a, 602 00 Brno, Czech Republic

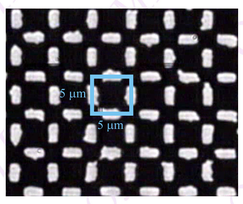
ABSTRACT

Tracing of foreign objects inside living cells is very exciting way how to study interior of living objects in non-destructive way. We imported fluorescent submicron particles into the living cells using liposomes as carriers to study the local mechanical heterogeneity of the cell cytoplasm. Thermal motion of these probes within the cell is tracked using fluorescent video-microscopy. The time-records of the probe positions reveal their trajectories and accessible space to the probes inside the cytoplasm of living cells. Further analyses of the thermal motion of the probes can reveal the mechanism of sub-cellular transport and properties of the cytoplasm in vivo .

METHODS

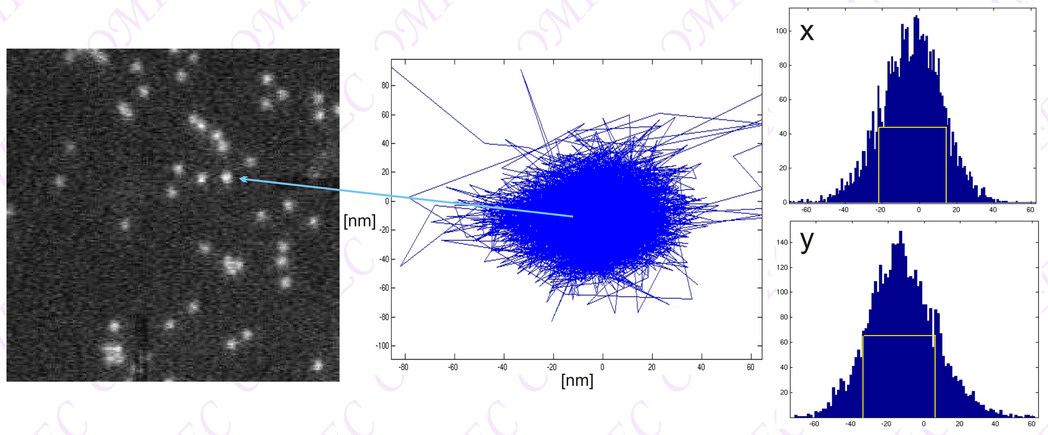
Cell culture - human adherent epithelial fibrosarcoma cells HT-1080 (ATCC number CCL-121) were cultivated in chambered coverglass (Lab-Tek II - 2 chambers, from Nunc, Denmark) with Minimum essential medium (MEM) without phenol red (tissue culture medium P04-00508 from PAN Biotech GmbH, Germany) supplemented with 10% fetal bovine serum (PAN Biotech GmbH, Germany).
Sample for liposomes - 10 µl of 300 nm fluorescent beads (Duke Scientific, 300 nm in diameter, fluorescently labelled) were diluted in sterile 240 µl of 1x PBS (phosphate buffered saline) with 1% bovine serum albumin to prevent sticking of fluorescent beads to cellular surfaces in later experiments.
Liposome formation and use - for liposome formation was used novel cationic lipid 1,2-dipalmitoyl-sn-glycero-3-ethylphosphocholine dissolved in chloroform (EDPPC) from Avanti Polar Lipids Inc. (U.S.A.). Prior liposome formation was 250 µl of EDPPC transfered into glass tube and dried by gaseous nitrogen. Sample containing fluorescent beads was pre-warmed to 45°C. Pre-warmed sample was then added into glass tube containing dried lipids and final mixture was vigorously vortexed and kept at 45°C for at least 30 minutes. Obtained multilamellar vesicles (LMV) formed by hydration were stored at 4-8°C. 15 µl of such prepared LMV were loaded into one chamber of chambered coverglass with cell culture and cultivated overnight together. Culture medium containing liposomes was exchanged for fresh medium on next day.
Measurement - cells cultivated in chambered coverglass was placed in optical microscope (Olympus IX70). Microscope stage was located in incubation box. Fluorescent lamp and CCD camera was used for recording of position of fluorescent particles. Duration of measurement was 10 min with frame rate 25 fps.
Data processing - recorded picture was processed by program Matlab using homemade code based on correlation algorithm .

Calibration



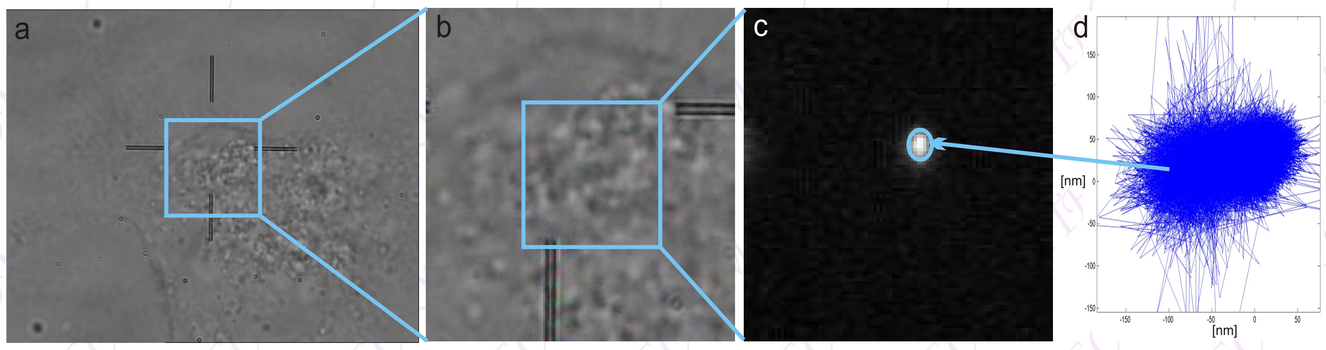
CCD camera was first calibrated using lithographic mask with spacing between grooves equal to 5µm. We obtained that one pixel corresponded to 62.5 nm using objective Olympus UPlanFl 100x/1.3 Oil Iri and 1/3` CCD chip (CCD camera Kampro KC-381CG).

Accuracy

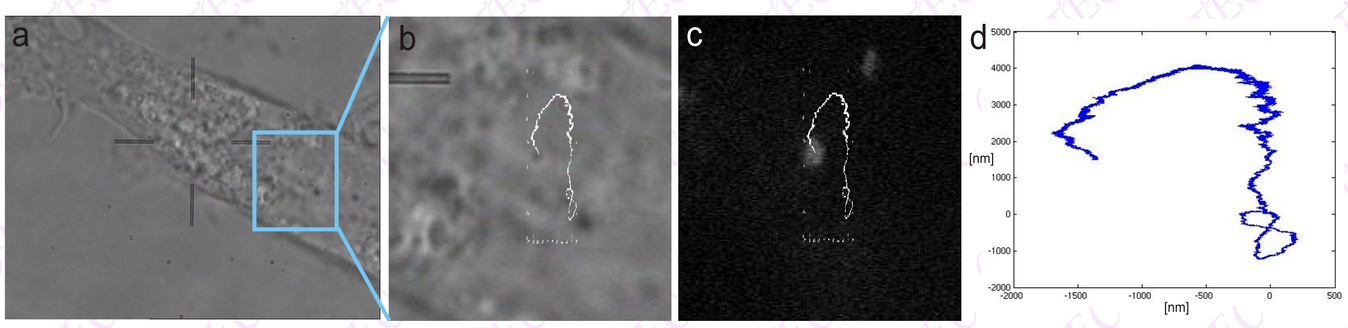


Accuracy of determination of particle position. Fluorescent beads of diameter 300 nm adhered to the coverglass (left). Processed time record of positions from a CCD camera for of a single bead (center). Histograms of particle positions in x and y axis (right). The FWHM of these histograms determines the uncertainty of the method.CCD pictures were processed by correlation algorithm and the accuracy of bead position determination was found to be 40 nm for both directions.

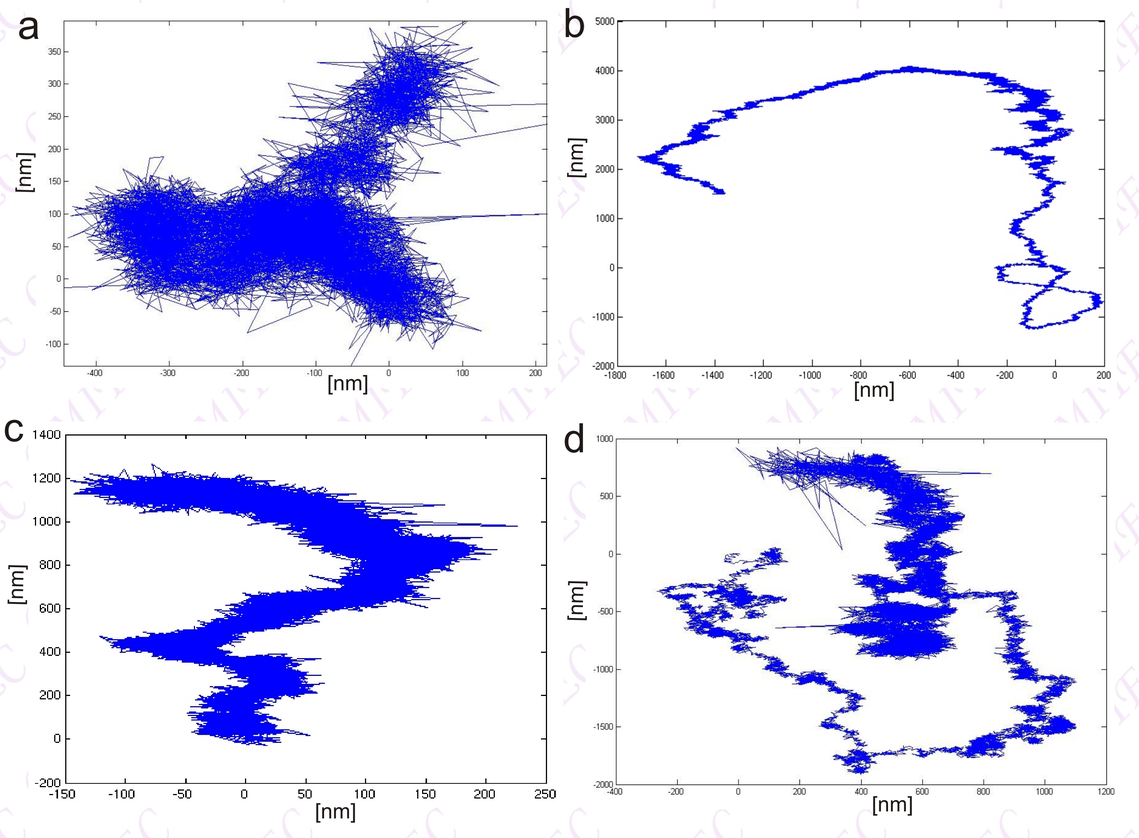
MEASUREMENT AND DATA PROCESSING



Example of the studied living cell with immobilized fluorescent bead inside. The fluorescent particles were not diluted in PBS with 1% bovine serum albumin and therefore they were adhered to sub-cellular structures frequently. **a** - all microscope field of view, **b** - detail of the cell, **c** - fluorescent bead inside the cell without bright field illumination and **d** - found bead positions from the record.



The particles were diluted in PBS with 1% bovine serum albumin. Therefore they were not able to adhere to the sub-cellular structures and so they moved freely inside the living cell. **a** - the whole field of view, **b** - detail of the cell in the end of recorded sequence with projected particle trajectory (white), **c** - fluorescence signal with particle trajectory and **d** - trajectory found from analyzes of video record.



Trajectories of four different particles inside living cell in different places. Part **a** shows a particle moving close to its original position, parts **b-d** show curvilinear motion inside the cell over several micrometers.

CONCLUSIONS

We studied the local mechanical heterogeneity of cytoplasm by observing the thermal and Brownian motion of particles in the cytoplasm of living cells. To import such particles into living cells we prepared lipid vesicles, liposomes, containing the particles in their inner space. Due to appropriate lipid composition, cellular membranes absorb the liposomes and their content is released into the cell cytoplasm. Our liposome preparation was limited to hydration technique of dried lipids with sample containing the particles. We didn't proceed with sonication or extrusion technique due to very large size of our fluorescent beads we wanted to encapsulate. However extrusion technique with polycarbonate filter of pore size 1µm may offer some improvement for transfer of particles into living cells and is being tested.
We show potential measurement of localization of foreign submicron particle in living cells with accuracy close to 40 nm. We have found that particles with untreated surface are adhered to cellular surfaces or structures frequently occurring inside the living cell. Therefore their motion is limited to thermal fluctuations around the place of fixation and at the same time to the movement of whole cellular structure within the cell. On the other hand, particles coated with bovine serum albumin showed no adhesion to cellular structures and moved freely along non-chaotic trajectories long several micrometers. If they stayed near the original position, they scanned accessible volume thus providing important information about local mechanical heterogeneity of the cytoplasm.

ACKNOWLEDGEMENT

This work was partially supported by the Institutional Research Plan of the Institute of Scientific Instruments (AV0Z20650511) and Grant Agency of the Academy of Sciences (IAA1065203)