MULTIPLE PROBE PHOTONIC FORCE MICROSCOPY

Petr JÁKL, Mojmír ŠERÝ, Pavel ZEMÁNEK

Institute of Scientific Instruments of the AS CR, v.v.i., Královopolská 147, 612 64 Brno, Czech Republic, EU,
jakl@isibrno.cz

Abstract

Single beam optical trap (also known as optical tweezers) is created by a laser beam that is tightly focused by microscope objective with high numerical aperture. A dielectric particle in water medium is then dragged by optical forces to place of the highest optical intensity, i.e. to the laser beam focus. Photonic force microscopy (PFM) is a technique that utilizes optical tweezers for confining the local probe, usually a dielectric particle of a sub-micron diameter. I.e. PFM belongs to the of large family of scanning probe microscopy (SPM) techniques. We have used fluorescently labeled polymer sphere in order to conveniently measure the distance between the particle center and the focal point of the laser beam. To make the measurement more precise, we have measured two-photon-fluorescence, which is quickly decreasing with the probe-focus distance. Standard PFM setup was enhanced by acousto-optical deflectors in order to generate two traps by a time-sharing technique – two probes were held in two different places by rapid switching of the trap positions. Therefore, the probes were not able to escape from the trapping region during short time of trap inactivity. By scanning the surface with probes separated several micrometers, we measured profile almost two times faster than using classic technique. We were able to obtain surface topography of microscope cover slip covered with objects as small as 60 nm in diameter.

Keywords: photonic force microscopy, scanning probe microscopy, two-photon fluorescence, time-sharing traps

1. INTRODUCTION

1.1 Optical trapping

Optical trapping is a popular technique widely used in medical science[1], microbiology or surface analysis[2-4]. It utilizes optical forces originating in a laser beam to confine or manipulate particles of sizes between tens of nanometres to tens of micrometres that are soluted in liquid medium (usually water). While the radiation pressure pushes microparticles in the direction of light propagation, the gradient forces originating in strong beam inhomogeneity tend to pull dielectric objects of higher refractive index than surrounding medium to places of the highest optical intensity. These forces reach upto hundreds of pN and they can be used to three-dimensional fixation of particle position.

The most popular experimental set-up is so-called single beam trap (SBT), where high gradient of optical intensity is created by a strongly focused laser beam with microscope objective with high numerical aperture[5]. In this case, the scattering and gradient forces create an equilibrium position located slightly beyond the focal point of the laser beam. For a slight deflection of the particle from this equilibrium position, forces can be considered as a Hookean spring and approximated by \( F_j = -\kappa_j \Delta x_j \), where \( \kappa_j \) is the trap stiffness and \( \Delta x_j \) is the particle deflection. Trap stiffnesses are generally different in each of the coordinate axes. Thus, with proper particle position detector, it is possible (after calibration of the optical forces) to use the SBT to perform precise measurement of piconewton-sized forces[6,7], or, in this application, to perform surface relief measurements. The main advantage of using optical forces instead of the mechanical cantilever for holding the probe is the possibility to measure surfaces which can not be accessed directly, e.g. hollows and cavities.
Position sensing can be performed many ways, including quadrant photodiode placed in back focal plane of the condenser lens[8], video particle tracking using pattern detection techniques[9], or mapping the fluorescent intensity[10]. The first two methods are slightly difficult to use in PFM, since we want to map reliefs of rough samples and the probe image or interference pattern are disturbed by the surface inhomogeneities. For such reason, we decided to use a fluorescently-bulk-dyed polystyrene sphere as a probe and a photomultiplier tube (PMT) as a position detector. Since we used the near-infrared laser to trap the probe and red fluorescent dye, the two-photon-fluorescence (TPF) took effect in this case. The distance between the probe and focus of the laser beam directly corresponds with the TPF intensity. Since the optical trapping requires strongly focused laser beam, the TPF signal (directly proportional to the square of optical intensity) drops fairly quickly with the distance from the focus. Utilizing this effect is more sensitive than employing single-photon-fluorescence for the task. The TPF position sensing does not distinguish between lateral and axial displacement of the probe from equilibrium position. However, the lateral forces in the SBT are about five times higher than axial ones in a common set-up, so the unwanted crosstalk can be considered negligible. The usage of this position detection method is limited by so-called photobleaching – irreversible photochemical changes to the fluorescent dye, which causes exponential decay of the TPF signal in time. Speed of scanning is therefore crucial parameter of PFM with TPF position detector.

### 1.2 Photonic force microscope

PFM is based on SBT with the probe position detection system that is completed with some sort of sample positioning mechanism. Because high precision movement of the sample is necessary, the most convenient devices are piezostages with some loop-back control, which can reach positioning repeatability in the order of units of nanometers. The probe is in contact with measured surface and pulled from the equilibrium position hundreds of nanometers away (but still held firmly in the optical trap). The axial distance between the probe and the trap changes with lateral movement of the sample, as the probe copies the surface relief. By recording TPF level, it is possible to measure this distance and, thus, the height changes of the sample surface. Since it is necessary to calibrate the position-signal function, three-dimensional movement of the sample is essential.

To omit the unwanted photobleaching effect, we have to minimize measurement time. One of the possibilities is to use two probes simultaneously and connect scanned areas during post-processing of experimentally obtained data. It is possible to trap several particles into traps created by laser beam divided with diffraction optical element or more sophisticated computer generated hologram. However, it is difficult to distinguish between position signals from particular probes by using these techniques. Therefore, we employed so-called time-sharing traps using acousto-optics deflectors (AOD). Position of the SBT is switched between two positions so rapidly, that the particle is not capable of escaping from trapping region by Brownian motion. The critical condition for switching frequency can be expressed as [11]

$$
\nu = \frac{1}{\tau} = \frac{2kT[erf^{-1}(\gamma)]^2}{3\pi \eta a^2 n^2},
$$

where $k$ is Boltzmann constant, $T$ is temperature of the medium, $a$ is probe radius, $\gamma$ is probability of particle not escaping further than $na$ distance from the trap for the time $\tau$, when it is not confined by the laser beam, and $\eta$ is viscosity of the water medium. In our case, sufficient repeating frequency was 200 Hz.

### 2. EXPERIMENT

#### 2.3 Description of apparatus

The apparatus was built around upright microscope Olympus BX50. Optical trap is created by strongly focused infrared beam coming out of all-solid-state Nd:YAG laser (Nd:YVO₄, Spectra Physics T20-B100-106Q; $\lambda$=1053 nm, maximal power 4W). The laser beam with diameter 2 mm is deflected by acousto-optical...
deflector (Isomet 1205C-2-804A) and expanded by two telescopes (2.75 mm x 8 mm and 60 mm x 180 mm) to overfill the entrance aperture of microscope objective. The objective is infinite distance oil immersion type with high numerical aperture (Olympus Ach 100x, N.A. 1.25 Ph). The measured surface is attached to the top side of the sample chamber filled with mixture of water, probes and spacers. Spacers are bigger polystyrene spheres (diameter 15 μm) that are used to define sample thickness. As a probes, we have used red dyed polystyrene microspheres of 200 nm and 820 nm in diameter (R200 and R800, Duke Scientific).

The red fluorescent has maximum excitation at 542 nm (nearly half of the trapping laser wavelength) and emits red light at 612 nm. The excited light is collected by the objective again and passes dichroic mirror, IR blocking filter and tube lens to PMT (R1527, Hamamatsu). Sample chamber is connected to three-axis nanopositioning stage P517.3C (Physik Instrumente) equipped with capacitive sensor. The sample position can be set with precision upto 5 nm laterally and 1 nm axially in closed loop operation. Whole experimental procedure is computer controlled. Piezostage was moved via 18-bit DA card (custom made at ÚPT AV ČR, v.v.i.), AOD and PMT were served by multifunction AD/DA card Computer Boards (PCI-DAS 4020/12). All the measurement software was written in Microsoft Visual C/++ to achieve maximal efficiency and speed of time critical operations.

2.4 Experimental procedure

The procedure is based on the single probe PFM measurement [12]. Two polystyrene probes are confined in two time-shared optical traps separated by 2.5 μm and they are in contact with the measured surface all the
time during scanning. In the beginning, two time-sharing traps are created by switching the diffraction grating on AOD with 200 Hz frequency. After trapping two probes into both traps, the probes are pushed from the equilibrium position more than 0.5 \( \mu \)m by the measured surface. Then, the measurement procedure follows, during which PMT signals from particular probes are strictly resolved. The measurement procedure can be summarized in following steps:

1. Vertical calibration. The sample is moved 500 nm vertically with 20 nm steps and TPF levels are recorded.

2. Circumference scan No. 1. The active region’s border is scanned with the probe for future elimination of photobleaching. Both TPF level and exact time is recorded in each point of circumference.

3. Area scan. The whole active region is scanned and averaged value from several tens of TPF values are recorded from each point.

4. Circumference scan No. 2.

After those steps postprocessing of the measurement takes place. The fluorescent photobleaching is supposed to have exponential character. Interpolated value of photobleaching constant is acquired for each point of measured surface and TPF value for each point of surface is re-calculated for the same time \( t_0 \). Finally, the vertical calibration is recalculated to \( t_0 \) and areas scanned by both probes are concatenated to the final surface profile.

2.5 Experimental results

![Fig. 2 Estimated resolution of the method. Series of 4 vertical scans with 1 nm step over 25 nm was performed and resolution better than 10 nm was obtained.](image)

![Fig. 3 Example of vertical calibration of the probe position to TPF signal level.](image)

![Fig. 4 Two circumference scans with the first probe. The photobleaching decay of TPF signal level is clearly visible.](image)
CONCLUSIONS

Scanning probe microscope with two optically held local probes was used to scan surface with submicron details. Simultaneous trapping was performed by time-sharing technique. The surface profile was obtained by measuring probes’ axial position via two-photon fluorescence excited by the trapping laser beam. This way of particle position detection is sensitive enough to get surface details with 10 nm resolution. The advantages of PFM over techniques employing mechanical cantilever is possibility to measure profiles of transparent surfaces without direct mechanical access (cavities) and surfaces of soft biological samples – the optical forces are two orders of magnitude lower than that of AFM probe. Disadvantage is that measuring probe is disturbed by Brownian motion which disables acquisition of surface topology with higher precision.

ACKNOWLEDGEMENTS

The authors would like to acknowledge support from the Institutional Research Plan of the Institute of Scientific Instruments (AV0Z20650511), the Ministry of Education, Youth, and Sports of the Czech Republic (projects No. LC06007) together with the European Commission (project ALISI No. CZ.1.05/2.1.00/01.0017)
LITERATURE


