The use of a optically trapped microprobe for scanning of surface details

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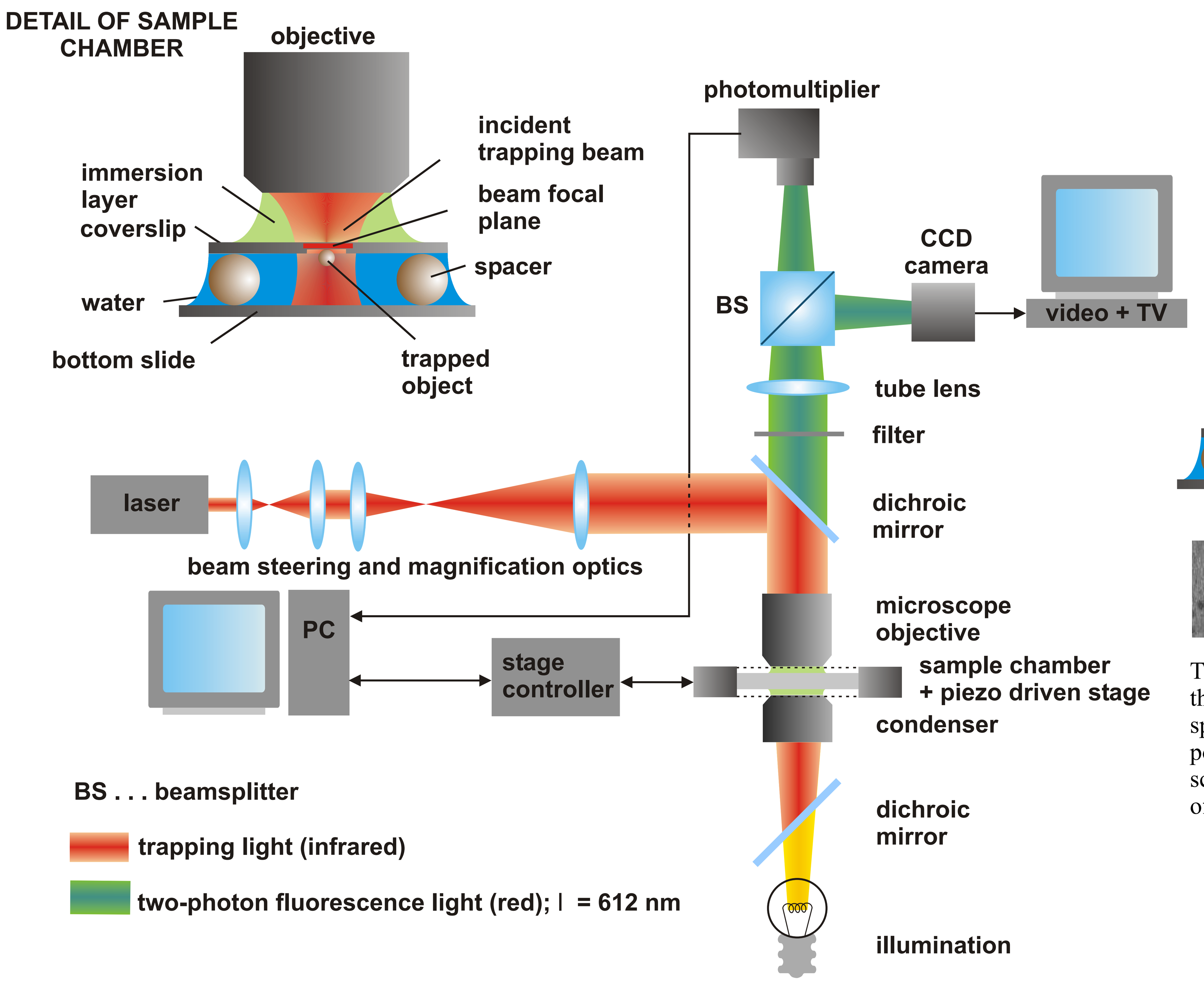
INTRODUCTION

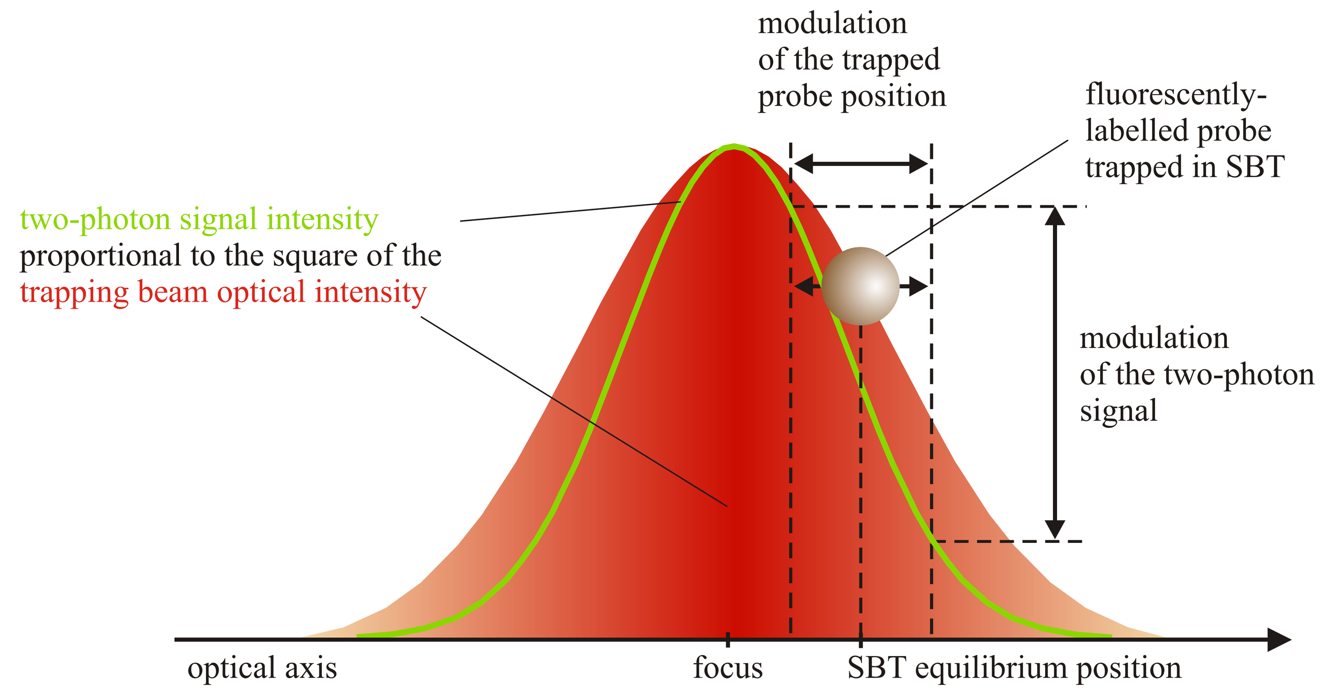
The probe is trapped by focused laser beam (so called optical tweezers) and the deviations of the probe from its equilibrium position are detected using two-photon fluorescence (TPF) excited by the trapping beam and emitted by the properly dyed probe (polymer microsphere). The optically trapped probe represents an analogue to the cantilever of Atomic Force Microscope (AFM) and therefore this method is sometimes called Photonic Force Microscope. The unique feature of the optical trapping is the possibility to confine a probe inside transparent objects (e.g. living cells) or behind transparent obstacles (e.g. coverslip). Therefore, there is no need for mechanical contact between the probe (trapped microparticle) and its holder (laser beam). We compare here results obtained by two methods of surface profile scanning- contact mode and tapping mode.

EXPERIMENTAL SET-UP



DETAIL	cover slip: Cellocate (Ependorf), spacers: polystyrene spheres, diameter 9.14 μm, (Polyscience, Polybead), trapped object: red fluorescent polymer microspher,es diameter 0.82 μm (Duke Scientific)
MAIN SETUP	Nd:YAG laser: DPY 321 II, max output 1 W, wavelength λ =1064 nm, (Adlas), photomultiplier tube: R1527 (Hamamatsu), objective: Ph3 100x, N. A. 1.25, oil immersion, (Olympus), piezo driven stage: 517.3C, capacitive sensors, position repeatability 5 nm, (Physik Instrumente)

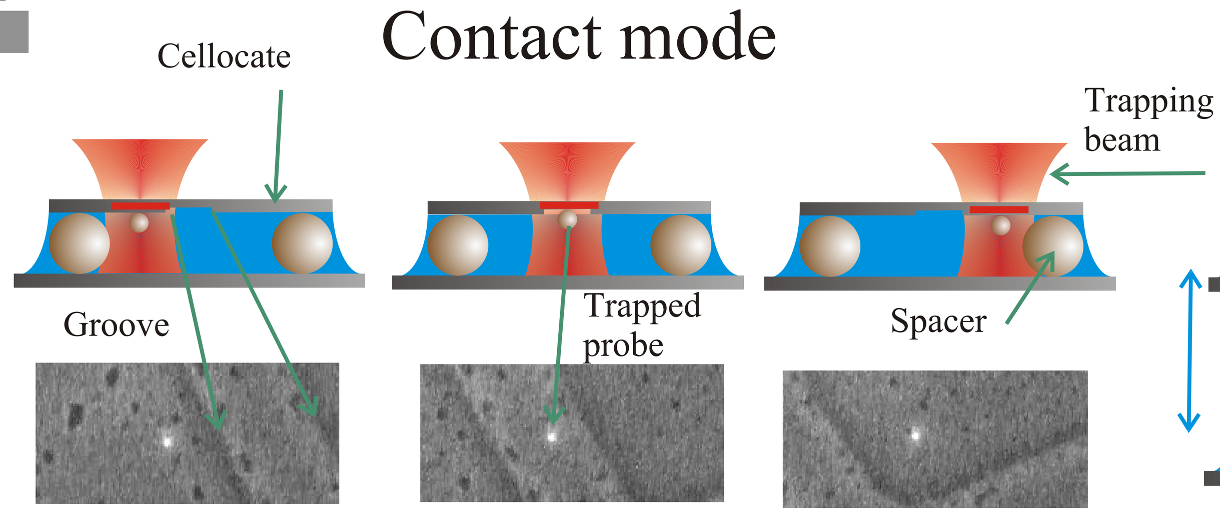
POSITION SENSING



Using two-photon excitation process, shift of the SBT-trapped fluorescently-labelled probe relative to the trapping beam focus can be measured with nanometer resolution. For small deviations of the probe from its SBT equilibrium position, the two-photon signal (TPS) emitted by the probe is approximately linearly proportional to the deviation.

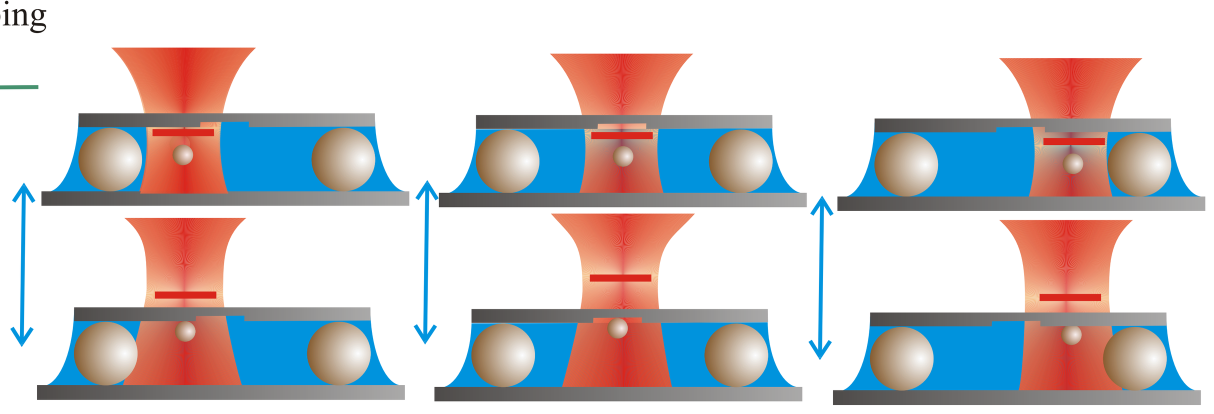
PRINCIPLES OF SCANNING MODES

Contact mode



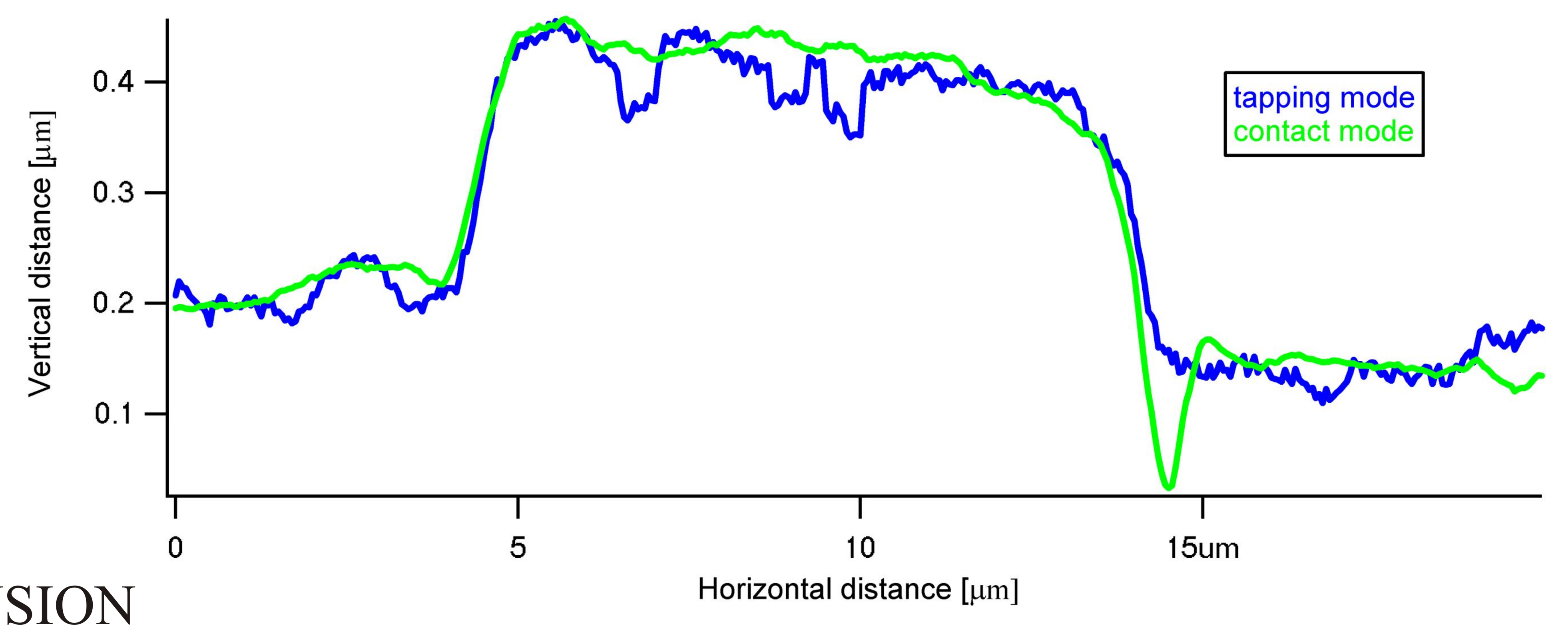
The probe is first trapped in 3D and than pushed against the studied surface so that the TPF signal decreases to 80%. The trapping beam works as a very soft spring that keeps the probe attached to the surface. The changes of the probe position due to the surface relief is detected as the changes in TPF signal. The scanning and TPF signal calibration is provided by the PZT stage. The bleaching of the dye is eliminated by repeated scans over the same surface region.

Tapping mode



At each lateral position the probe is first trapped in 3D about 1 micrometer below the Cellocate and than pushed against the studied surface. The vertical distance that is needed to reach the 80% level of TPF signal, is recorded and the surface profile is then reconstructed from these values. This method eliminates bleaching of the dye, does not need calibration of the TPF signal but it is much slower.

EXPERIMENTAL COMPARISON



CONCLUSION

We demonstrated two simple methods of local probe microscopy with optically trapped probe which provide resolution in the order of tens of nanometers and which are useful for the study of surface profile inside transparent objects. The vertical sensitivity of contact mode and tapping mode methods was determined to 25 nm. Good coincidence between both methods is found in the lateral direction (groove width) and axial direction (groove depth). Worse coincidence is found in the details on the surface because the TPF position sensing detects not only the vertical displacement of the probe, but also lateral probe displacement with respect to the intensity maximum. Complete 3D position sensing will improve the accuracy of both methods.

ACKNOWLEDGMENTS

This work was supported by the Grant Agency of the Czech Republic, projects No: 101/00/0974.