# HAPTIC THREE-DIMENSIONAL INTRACELLULAR NANOMANIPULATOR

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Classical AFM based nanomanipulator may allow movement of objects on a surface, however is lacking ´pick and release´ function.

We have built a fully interactive three-dimensional (3-D) nanomanipulator integrated with an optical trapping-based Photonic Force Microscope (PFM) for in-vivo and in-situ operation inside living cells. A PFM is combined with a 3-D real-time haptic (force feedback) nanomanipulator. The system is based on an optical trap with an ultra-fast 3-D position detection system. It allows to manipulate in 3-D a probe inserted inside a living cell and to record its position with nanometer spatial and microsecond temporal resolution. By mapping the thermally induced movements of the probe, it is possible to measure intracellular forces and the 3-D stiffness matrix in real time. This instrument allow interactive accessing, positioning, measuring, imaging, and reacting.

The protocol of introducing 240 nm polystyrene beads inside a cell was elaborated. It enables to place beads in the cytoplasm within 12 hours of incubation of cells with the solution containing few probing beads. The incubation time is dependent on the bead size, surface charge, coating. The identification of beads inside cells is realized by the fluorescence optical chain that was added to the system.

The chosen biological model for probing cell interior was based on two cell lines from human bladder: non-malignant cells of urether (HCV29 cell line) and malignant bladder cells (T24 cell lines). Together with PFM measurements, the characterization of cell interior has been performed using TEM (transmission electron microscope) and fluorescence microscope in order to get information on the spatial distribution of a cell cytoskeleton and cytoplasm within cell interior.

The first results of cell interior probing showed similar values of stiffness inside cells but the character of its distribution was different depending on the cell type. To characterize the differences in a quantitative way, the parameters describing the stiffness anisotropy has been delivered. It showed that the probing beads have more space to fluctuate in cancerous cells while in nonmalignant cells the beads fluctuates along a defined direction.

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