


# Manipulation of biological cells by AFM

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**Abstract**

Manipulation by nanorobot is a novel topic about controlled manipulation of nano meter sized objects and reactions between objects in atomic and molecular sizes. The ability of AFM for measuring the forces in Nano Newton range in physiological condition and liquid environment has been made it a very practical instrument in the investigations related to biological applications such as interactions between drug-protein, protein-protein, cell-cell, cell-protein, etc.
Until now some investigators worked on dynamic modeling of nanomanipulation and achieved satisfactory results. Most of the researches have been conducted in gas environment. But in the case of biological conditions, the environment is frequently liquid with diverse properties. In this article, the environment is assumed to be biological liquid and due to several forces applied in this environment, different methods of manipulation and simulation of manipulation of biological cells have been investigated. Results have been compared with previous works and their applicability is discussed.

**Key words:** Nanomanipulation, AFM, Biological condition, Cell interaction

## 1. Introduction

Nanomanipulation is as an emerging area which enables to modify, interact and control at Nano scale accurately and has received enormous attention for the last few years. Applications of the nanotechnology can be addressed in numerous fields such as biotechnology (DNA and protein study), data storage in Nanomaterials or materials science (Nanotube or surface film characterization). In contrast to what happened for the majority of the microscopy techniques (as well as for several other experimental techniques), lack of reproducibility of the preceding outcomes and the disparities of the conclusions of some of these primary studies have dimed the light to corroborate the applicability of this technique on the study of samples when biological studies are intended. Previous doubts were certainly eradicated by Bustamante et al. (1992), with the publication of the first reliable DNA images obtained by AFM. Since then, the monumental ameliorations were accomplished both at the instrumental and sample preparation stages, led to a fast development of the atomic force microscopy of biologically relevant samples. In order to meet the increasingly diversifying need for the cell manipulation in the boosting progress of cell engineering, Sumaru et al. (2007) developed a novel technique to capture the living cells on a culture substrate by irradiating light in a intermittently. After the light irradiation in arbitrary micropattern by using a newly developed apparatus and the process to remove non-captured cells including EDTA treatment, the highly contrasted cell patterns were formed with the precision of single cell size. It was attested that the cells maintained their viability well after the manipulation process including photo-induced cell harvesting. Sitti (2000) contemplated surface forces using JKR theory and propounded a new model for tele-operated nanoparticle pushing. More comprehensive pushing dynamic model was proposed by Taffazzoli and Sitti(2004). Korayem and Zakeri(2009) have investigated manipulation models and developed it for sensitivity investigations of pushing critical conditions in AFM-based nanomanipulation, including the nanoparticle pushing force and time versus changing all parameters of the nanomanipulation process and their corresponding model comprising both adhesional and normal friction forces. Also, pull-off forces are modeled by using the Johnson– Kendall–Roberts (JKR) contact mechanics model. Korayem et al.(2009) focused on submerged manipulation in liquid and analyzed the artificial nano robot manipulation applying theoretical forces analysis. Regarding to the importance of manipulation modeling in liquids, the applied hydrodynamic forces on cantilever is extracted and the new dynamic model is simulated for manipulation of submerged spherical nanoparticle. Significance of distance, contact and hydrodynamic forces were investigated and then a newly proposed dynamic modeling of gold particle manipulation on a silicone substrate was conducted in liquid. Applications of the conventional AFM succeeded in manipulating nanoparticles, nanowires or nanotubes by extensively used pushing or pulling operations on a single plane. However, pick-and-place nanomanipulation is still a challenge in the air. Xie et al. (2009) developed a modified AFM, called three-dimensional (3D) manipulation force microscope (3DMFM), aiming to achieve the pick-and-place in the air. This system in essence is made up of two microcantilevers and each is equipped with a nanopositioning device and an optical lever, forming a nanotweezer with capabilities of picking and releasing nanoobjects with force sensing. Before the appearance of 3D manipulation, one of the cantilevers is employed to position nanoobjects and locate the tip of another cantilever by image scanning, then these two cantilevers fit together as a nanotweezer to capture, transport, place and even supersede the nanoobjects with real-time force sensing. Using AFM probe as a manipulation tool enables precise positioning particle for micro/nanoassembly, which is the essential element for accurate control of nanoparticles positioning and assemblies. Nanomanipulation approaches are grouped and listed in table 1. Nano-object manipulation based on AFM is one of the dominant means for constructing miniaturized system and machines.

| Starting Point-Based | Process-Based | Interaction Type-Based | Operation-Based |
|---|---|---|---|
| Top-down | Self-assembly | contact | Teleoperated |
| Bottom-up | physical | Non-contact | Semi-autonomous |
| | | | automatic |

Table 1. Nano Scale Object Manipulation Approaches

For efficacious simulation of mechanic and dynamics of nanoparticle pushing by probe of AFM, at first physical definition of the problem in nano-scale are outlined. Passing from the macro to the nano world, the major phenomenon is reduction of the object's size; and the impact of this change in the length is defined as the scaling effect, which leads to some changes in geometrical and physical properties. When the size of object is less than 1 μm, adhesion forces become dominant with respect to the inertial forces in object interactions as a problem definition in this paper, the dynamics of nanomanipulation system, including cantilever and probe of AFM, nanoparticle, and substrate is simulated.
Therefore, until now the researches have been conducted in gas and liquid environments. But in the case of biological conditions, the environment is frequently a biological liquid with diverse properties. In this article, the environment is assumed to be a biological liquid and due to several forces applied in this environment, different manipulation methods and simulation of manipulation of biological cells have been investigated.

## 2. Research Methodology

The manipulation process cannot be observed in real time. During the pushing of the objects, imaging is impossible because the same tool that should be used for the imaging is the manipulation tool. As a solution, surfaces that are subjected to particles could be imaged before and after manipulation, and applying the taken images, relative position of particles to the basic reference point is determined. Two distinct methods can be considered for pushing nanoparticles in constant velocity: (1) Moving the substrate while AFM probe is in contact with particle; (2) AFM probe tip moves and pushes the targeted particle on the immobile substrate. Eventual dynamic results for either method would be similar. The second method is used in this paper where the probe forces $F_T$ acting between the tip and the particle is kept constant during nanoparticles movement on substrate.
In liquid environments, there are some additional forces involving molecular and hydrodynamic forces. Micro/nano scale interactions include molecular forces such as van der waals, electrostatic double layer, steric, salvation/hydration forces as well as surface tension. Electrostatic Double Layer force arises between charged surfaces. When a surface approaches to another, the double layer is perturbed; the resulting force is recognized as ESDL. At large distance, this force decays almost exponentially. Surface charge depends on pH and salt concentration. Steric force happens between surfaces with adsorbed polymer layers or across polymer solution. At short ranges, oscillatory salvation forces arise whenever liquid molecules are induced to order into quasi- discrete layers between two surfaces of within a highly confined space. In aqueous solutions, between hydrophilic surfaces the force becomes exponentially repulsive and is known as hydration force. The dynamic equations in y and z directions and torsion are obtained by using the equilibrium equations. The forces are shown in Fig 1.

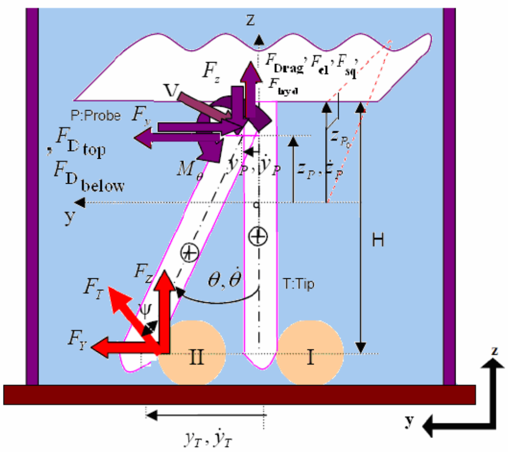

**Fig 1**: Free body diagram of AFM probe

$$F_Y = (F_y - F_d)\sin^2\theta - (F_z - F_{el} - F_{sq} - F_{hyd})\sin\theta\cos\theta + \frac{m}{2}\ddot{y}_P \sin^2\theta + \frac{(I_C\ddot{\theta} + M_\theta)\cos\theta}{H} - \frac{m}{2}\ddot{z}_P \sin\theta\cos\theta \qquad (1)$$

$$F_Z = \frac{M_\theta}{H}\sin\theta + \frac{I_C}{H}\sin\theta\,\ddot{\theta} + \cos^2\theta(F_z + \frac{m}{2}\ddot{z}_P - F_{el} - F_{sq} - F_{hyd}) + \frac{1}{2}\sin 2\theta(F_d - F_y - \frac{m}{2}\ddot{y}_P) \qquad (2)$$

$$F_T = \sqrt{F_Y^2 + F_X^2}, \psi = \tan^{-1}(\frac{F_Y}{F_Z}) \qquad (3)$$

In this study, models are simulated by Mathematica software and JKR contact theory is used in the formulation. There are 5 steps for manipulation of nanoparticles. In the first step the probe of microscope starts to move in the air and it reaches the particle in the second step in the liquid. Then it starts pushing it in the third step and moves during the fourth step. Finally the probe comes back to the first position in fifth step.

**3. Results and Analysis**

There are no differences between manipulation of nanoparticles and cells in the first three steps as there is no interaction yet. The changes appear in the forth step where the probe begins pushing the particle. As pushing launches, the flexibility of the cell leads to unfamiliar changes in the figures which can make difficulties and even cause the process to stop.

Diagrams of each step are shown in this section which are the same at first for manipulation of cells and nanoparticles. The results are compared for the forth step.

There are neither forces from the liquid nor contact depth and radius between probe and particle in the first step. (Figs 2, 3)

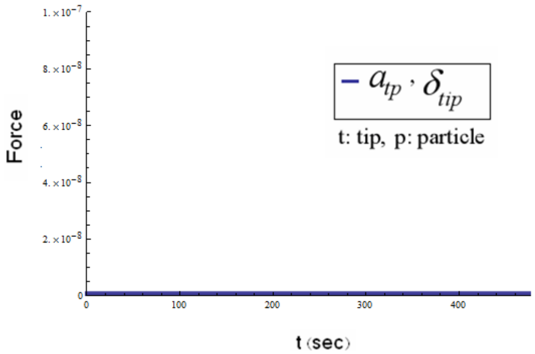


**Fig 2**: No depth and contact radius in first step

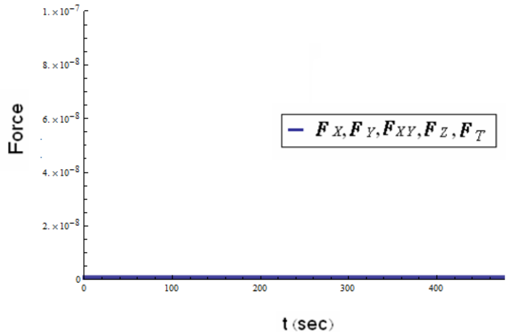


**Fig 3**: No forces in first step

The cantilever moves in the air to approach the liquid in the first step. Changes in 2 directions, X and Y, are shown in fig 4.

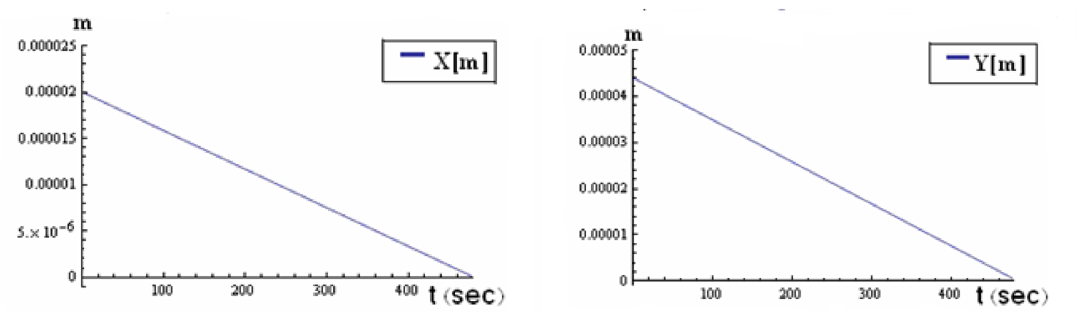


**Fig 4:** Displacements of probe in X and Y direction

The resultant force on the probe during the process is shown in fig 5.

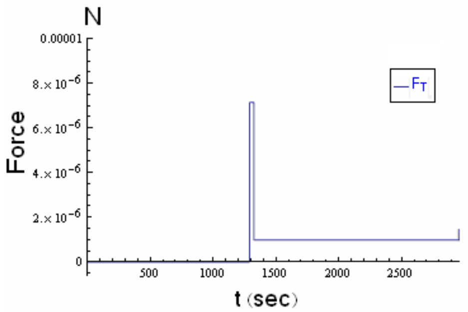


**Fig 5:** Resultant force during second step

Increasing the depth and contact radius during the fourth step shows that there is no sliding between probe and nanoparticles so manipulation can be done successfully. (Figs 6, 7)

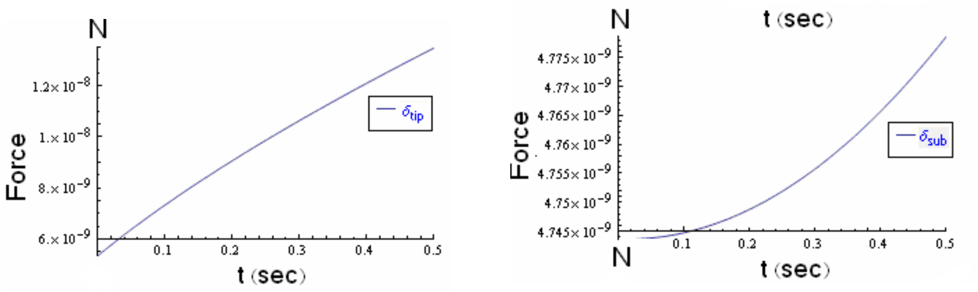


**Fig 6:** Contact depth changes during forth step for nanoparticles

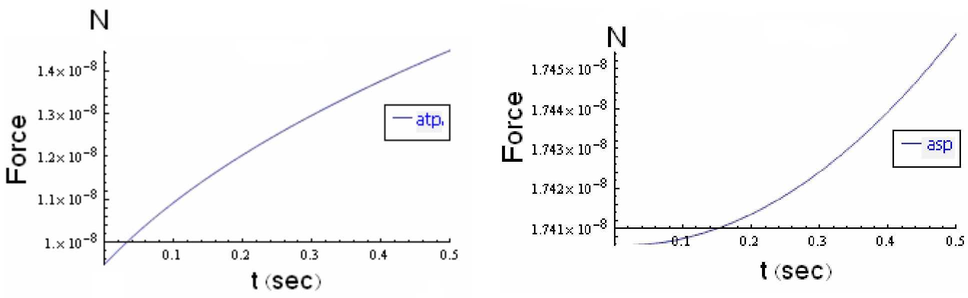


**Fig 7:** Contact radius changes during forth step for nanoparticles

But since biological cells have much smaller elasticity modulus than nanoparticles, diagrams of this step for their manipulation are different. Having constant depth and contact radius shows that in this case we have sliding; therefore, obviously JKR theory is not valid for cells. (Figs 8, 9)

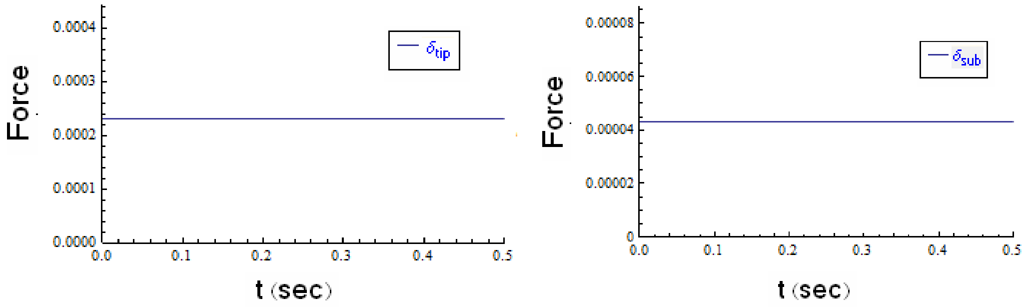


**Fig 8:** Depth changes during forth step for cells

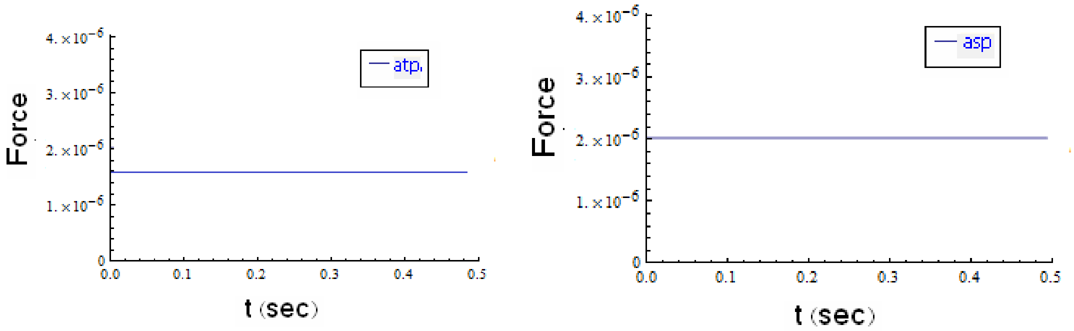


**Fig 9:** Contact radius changes during forth step for cells

## 4. Conclusions

In this paper manipulation of nanoparticles, especially biological cells in liquid, has been investigated. The contact theory assumed to be JKR, but due to small elasticity modulus of cells, the results show that another theory must be used to manipulate cells. While JKR theory is mostly used for small deformations, there are large deformations in cells. There are other models which used for deformations in large scales. A simple one is Tatara model in which adhesion force is not considered and it has the same contact geometry of Hertz model. One of the future works which can be studied is biological cell manipulation with the use of Tatara theory.

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