Sensitivity Analysis of the Critical Conditions of AFM-Based Biomanipulation of Cylindrical Biological Particles in Various Biological Mediums by Means of the Sobol Method

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Abstract

The sensitivity analysis of atomic force microscope (AFM) based manipulation of gold spherical nanoparticles in air medium has been carried out in previous research works. In the AFM-based manipulations conducted in various biological liquid mediums, the new environmental parameters associated with these biological fluids also affect the dynamics of the manipulation process. Therefore in this research, the Sobol sensitivity analysis method has been employed to find out how these new parameters as well as the other effective parameters influence the manipulation process. The parameters have been classified into two groups of AFM parameters and environmental parameters. According to the obtained simulation results, in the group of AFM parameters, cantilever thickness (with a sensitivity index of 57%) is the most sensitive parameter in the manipulation of cylindrical biological micro/nanoparticles, followed by the parameters of cantilever length and cantilever width. By examining the sensitivity of environmental parameters for cylindrical biological micro/nanoparticles in biological mediums, it is observed that the highest sensitivity belongs to the dimensional parameters of target particles (the most sensitive environmental parameter is cross-sectional radius of target particle with a sensitivity index of 52%), followed by the sensitivity of adhesion work in the biological medium of manipulation. It is found that the critical force for the onset of movement increases substantially with the increase in the cross-sectional radius of target particle, ratio of particle length to its cross-sectional radius and also with the increase in the work of adhesion in the biological environment.

Keywords: Sensitivity analysis, Biomanipulation, Atomic force microscopy, Different biological mediums, Cylindrical biological particles.

1. INRODUCTION

In recent years, the atomic force microscope, as one of the most accurate and flexible instruments for the 3D imaging, manipulation and analysis of material structures, has found extensive uses in many applied research works in engineering fields and biological sciences. The AFM has played a significant role in identifying the structural characteristics and mechanical properties of biomaterials and in the manipulation processes involving the displacement of particles on the surfaces and interfaces of these materials.

One of the most prominent features of AFM, which has led to the extensive use of this instrument in biological researches, is its ability to function in different environments including vacuum and air and in liquid mediums such as different types of biological and physiological fluids. This characteristic of AFM makes it

possible to conduct research in the natural living conditions of various biological species including living cells and proteins.

Cellsurgery procedures and manipulations of single or multiple cells, known as biomanipulations, take place in a vast range of applied research in the fields of biotechnology and cell biology. The first controlled nanomanipulation of biomolecules was carried out on genetic materials using the AFM [3]. In successive research works by Henderson et al. and Hansma et al. isolated strings of DNA were adsorbed on a surface of Mica and these strings were cut by an AFM probe in air and Propanol mediums, respectively [7,4, 5]. One of the preliminary researches in this field is the theoretical and experimental researches by Falvo et al. This group attempted to use the AFM tip to manipulate cylinder shape tobacco mosaic viruses (TMVs) on substrates made of Mica and graphite. In the first case, these researchers did not succeed in the pushing and deforming of TMV particles without cutting them, which they attributed to the high adhesion between these particles and the substrate made of Mica. In the second case, they succeeded in operations such as the cutting, rotating and displacing of particles on the graphite substrate. They also presented a mechanical model for describing the behavior of TMV particles during the manipulation process [2].

Li et al. employed the AFM to acquire images of live nerve cells and DNA molecules. Then, they used the virtual reality system and the AFM probe to displace the nerve cells. They inserted the AFM tip into the cell membrane and cut the cells at specific locations. They also deformed the DNA molecules and moved them on the substrate [21]. In a similar experimental research using the AFM, Lu precipitated and extended single DNA molecules on a substrate made of reformed Mica. He then imaged these molecules using the AFM vibration mode and performed cutting, bending and pushing operations on these particles [22]. In an

investigation conducted by Stark et al., human chromosomes were dissected by AFM under ambient conditions and in a buffer solution, and the effects of different types of buffer solutions on the swelling and elastic behavior of chromosomes were studied [28].

In a research, with the goal of achieving a high accuracy in the manipulation of cells and the analysis of the manipulation process, Han et al. introduced a technology called "Cell Surgery" through which, materials can be directly transferred into or taken out of a cell. This technique is very useful in the effective transfer of genes. In this technology, very thin needles are inserted into live cells by means of the AFM. In this investigation, the researchers fabricated cylindrical and conical nanoneedles made of Silicone and diamond and studied the effect of their size on the insertion efficiency. Also, to prove the effectiveness of diamond nanoneedles, they used an amine-modified diamond nanoneedle to deliver DNA material to mouse embryo fibroblast cells [6]. Hong et al. also investigated different manipulation modes such as cutting, pushing, moving on particle surface, bending and folding, indenting, lifting, etc. for the DNA molecules [8].

Following the preliminary model for the pushing of nanoparticles, which was presented by Falvo and which did not account for the effect of forces in the manipulation process, Sitti presented the first dynamic model of the process by considering the surface forces [25]. In another research, after studying the process more thoroughly, Sitti and Tafazzoli explored the different possible dynamic modes for the process [29]. Korayem et al. presented a theoretical analysis of the manipulation of spherical nanoparticles in liquid medium by means of the AFM. They evaluated the differences between manipulations in air and liquid mediums, and by adding the influences of the hydrodynamic surface forces to the forces existing in air medium, they developed a

model for the manipulation of spherical particles in liquid environment. In their research, the process of pushing gold nanoparticles with a radius of 50 nm and at a constant speed of 100 nm/s on a Silicon substrate has been simulated, the dynamic behaviors of AFM tip and nanoparticle have been investigated and the simulation results have been compared with the existing results for air medium [9]. Moradi et al. presented a new dynamic model for the manipulation process of pushing flexible nanorods on an elastic substrate, in air medium, by the AFM AFM tip [23]. In another recent research work, with the aim of making the previous dynamic models more accurate and complete, Korayem et al. developed the dynamic model of the AFM-based manipulation process of cylindrical micro/nanoparticles considering the existence of roughness on the surface of target micro/nanoparticles and their contact with smooth surfaces. In this study, the process is also simulated for different particle sizes and various roughness dimensions and based on the simulation results, the critical force and time of manipulation diminish for rough particles relative to smooth ones [10].

Due to the substantial complexity of the AFM-based dynamic manipulation models, a large number of parameters and inputs with different levels of sensitivity need to be identified for these models so that the process can be designed and implemented appropriately. Since there are a lot of input parameters that influence the critical force and time of movement and since the relevant equations are totally coupled, the controlling of the critical manipulation output values is essential and the determination of the parameters with the highest influence on output variables is very important in the manipulation process. By employing the graphical sensitivity analysis approach, for the first time, Korayem and Zakeri analyzed the sensitivity of the parameters related to the AFM-based manipulation of nanoparticles. Their nanomanipulation model simulates the critical force and time for the pushing of nanoparticles based on the variation of all the process parameters, and it uses the JKR contact mechanics model for the contact of gold nanoparticles with Silicone substrate and probe [12]. Korayem and Taheri have been developed the cylindrical contact models and then the circular crowned roller contact models and compared with the spherical contact model of Hertz. Then, they have been simulated these models in bioenvironments for the manipulation of different biological micro/nanoparticles which have been assumed as nanorods and circular crowned nanorollers [14].

In another work, Korayem et al. employed sensitivity analysis to investigate the use of advanced friction models in the manipulation of nanoparticles. Their graphical sensitivity analysis results indicate that, relative to the Coulomb model, HK and LuGre models achieve lower values of the critical force and time of movement. The critical force needed for the onset of movement diminishes by 15.87% in the HK model and by 22.22% in the LuGre model and also the critical time for the onset of movement diminishes by 50% in the HK model and by 75% in the LuGre model, relative to the Coulomb model [13].

Also, Korayem et al. applied the Sobol method to analyze the sensitivity of the spherical nanoparticles manipulation parameters for the frictional model of LuGre. The obtained results indicated that the dimensional parameters of cantilever are the most sensitive parameters in the manipulation process [11]. In addition to nanoparticle manipulation dynamics, sensitivity analysis is used in other fields such as study of flexural vibration for AFM cantilever with a crack and investigation of lateral field excited acoustic wave gas sensors with finite element method [1, 20].

In this study, a dynamic model for the AFM-based biomanipulation of cylindrical biological particles has been presented by

making necessary changes in the model in order to develop it for the process of manipulation in biological and physiological mediums. Then the sensitivity analysis model used in this research has been described. Subsequently, in order to investigate the effects of geometrical AFM parameters and environmental parameters used in the dynamic model of the AFM-based biomanipulation process on the critical force and time of manipulation, several dynamic simulations have been performed for cylindrical organic micro/nanoparticles in various biological environments. Finally, the results achieved by these simulations have been discussed.

Fig. 1 indicates the main AFM components involved in AFM based manipulation process; the manipulation process in this study is by means of pushing method and it is started with exerting force on the target particle by means of AFM tip. Further explanation regarding the process is provided in modeling section.
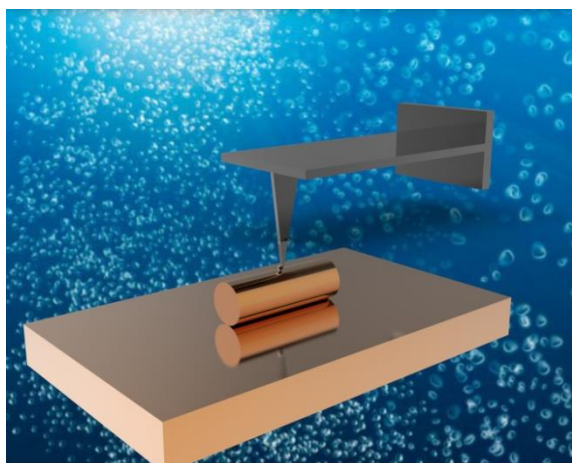

Figure1. *Main AFM components which are involved in AFM based manipulation process.*

2. METHODS AND MODELING
2.1. The model for the manipulation of organic particles in biological mediums
In recent years, the atomic force microscope has been used in numerous biological research works as a manipulator for performing displacement as well as other operations. The AFM-based manipulation process of a particle begins with the movement of substrate at a constant speed (V_{sub}), which causes the particle stuck on the substrate to move and brings it into contact with the AFM tip. As a result of this movement, the forces applied on the particle and the reaction force on the AFM tip start to increase in magnitude. This reaction force produces bending and twisting in the AFM cantilever. With the increase in the deformations of cantilever and the reaction forces exerted on the particle, the particle starts to move on the substrate. Therefore, the forces applied on the probe by the environment in which the AFM operates are very influential on dynamics of the process. Since many biological research works conducted on organic species are sensitive about not damaging the delicate samples that are studied, an exact knowledge of how different factors affect these manipulation processes and the development of a precise dynamic model for them become particularly important.

In the past research endeavors, dynamic models of manipulation have been presented for the pushing of spherical particles by the AFM, and in a limited number, for the pushing of particles with non-spherical geometries in air and liquid mediums and also simulations have been performed for particles of different dimensions and for certain organic particles such as DNA in air and water mediums, and in some substitute fluids like alcohol and blood [12, 13, 25].

Many organic particles tend to have a more cylindrical shape geometry rather than a simpler and more general spherical geometry. Some of these particles, like certain bacteria and viruses, have a limited length which is comparable to their cross sectional radius. Conversely, the single strands of DNA particles can be considered as a cylinder whose length is much larger than its cross sectional radius. Also, particles with a disk shape geometry, such as platelets, whose lengths are much

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shorter than their cross sectional radius, can be included among this class of cylindrical particles.

Since water forms the basis of many biological and physiological fluids, in the modeling and simulation of liquid environments, water is considered as the main liquid medium. Another frequently used liquid medium is alcohol. Blood and blood plasma are the other working environments of interest in medical research due to the large number of important organic particles that exist in them. Laboratory culture solutions are the other widely used liquid environments in biological research. Among these liquid mediums, one can mention the PBS solution, which is an extensively used buffer solution in biological research, and the DMEM solution, which is used in culture solution mixes and in pharmaceutics.

Properties of the working environment of AFM during the manipulation process, such as viscosity, properties that affect the hydration and electrostatic forces, and the adhesion properties all influence the dynamic behavior of the target particle in the course of manipulation; therefore, the effects of these parameters on the critical force and time of manipulation have been explored in this research. One of the most important of these properties is the adhesion work, which directly depends on the surface energy property (γ_i) of the manipulation environment and the target particle. The values of the work of adhesion in the biological environment between particle-AFM tip and particle-substrate in different biological mediums have been calculated by means of Eqs. 1 through 4 and the surface energy values of particle and the chosen medium for manipulation. These values of the work of adhesion in the biological environment are then substituted into the JKR cylindrical contact theory equations and used in the dynamic equations of the process. Finally, in simulating the sensitivity analysis of this important property, the interval of changes

is so chosen that the values obtained from the calculations related to various biological mediums(which some of these values have been presented in Table 1) can be covered.

When two materials are in contact with each other, the energy of their joint surface (per unit area) is expressed as mutual energy or mutual tension (γ_{12}) and is obtained as follows [19]:

$$\gamma_{12} = \frac{1}{2}W_{11} + \frac{1}{2}W_{22} - W_{12} = \gamma_1 + \gamma_2 - W_{12} \qquad (1)$$

The relation between γ_{12}, γ_1 and γ_2, from a thermodynamic standpoint, is presented as[19]:

$$\gamma_{ij} = \gamma_i + \gamma_j - 2\sqrt{\gamma_i \gamma_j} \qquad (2)$$

Thus, based on Eqs. 1 and 2 we have:

$$W_{12} = \gamma_1 + \gamma_2 - \gamma_{12} = 2\sqrt{\gamma_1 \gamma_2} \qquad (3)$$

However, to separate materials 1 and 2 which are immersed in medium 3, the adhesion work is obtained from the following equation:

$$W_{132} = W_{12} + W_{33} - W_{13} - W_{23} = \gamma_{13} + \gamma_{23} - \gamma_{12} \quad (4)$$

In previous research works, the model of manipulation in air medium has been extended to the liquid medium and the effect of drag and surface tension forces applied on cantilever has been considered as the demarcation line between the dynamic models of the manipulation process in liquid and air mediums; and these forces, as part of the externally applied forces, have been substituted into the equations of JKR contact theory [12]. In the following, the kinematic and dynamic equations pertaining to the manipulation of cylindrical micro/ nanoparticles in biological environments have been presented based on the equations developed in previous works.

Considering the changes carried out in previous research works, the horizontal deformation of the AFM tip is equal to:

$$y_P = \frac{F_y}{K_y} + \delta_D + \delta_{St} \qquad (5)$$

So, we will have:

$$F_y = K_y(y_p - \delta_D - \delta_{St}) \qquad (6)$$

Where δ_D and δ_{St} denote the cantilever deformations due to the drag and surface tension forces in liquid medium, respectively, K_y is lateral spring constant of the AFM cantilever, F_y is lateral force of the cantilever and y_p is the horizontal deformation of probe. These parameters are calculated according to Eqs. 7 and 8.

$$\delta_{St} = \frac{F_{St}L_1{}^3}{3E_{AFM}IM} \tag{7}$$

$$\delta_D = \frac{F_D(L-L_1)^3}{8E_{AFM}IM} \tag{8}$$

Where E_{AFM} is the AFM modulus of elasticity, IM, L is cantilever length. F_{St} and F_D in the above equations are calculated as follows [12]:

$$F_{St} = -\gamma b \tag{9}$$

$$F_D = 2(WL)\mu_v \frac{V_{sub}}{H} \tag{10}$$

Where b is the perimeter of the AFM cantilever, where it contacts the surface of the liquid. The constant γ is called the surface tension. W and L are the width and the length of the AFM cantilever. μ_v is the viscosity of fluid. H is the gap between the beam and the substrate and V_{sub} is the velocity of substrate.

Also, the kinematic equations of the manipulation process are expressed as Eqs. 11 and 12.

$$y_P = y_{sub.} + (R_p - \delta_{tip})\sin\varphi - H\sin\theta \tag{11}$$

$$z_P = z_{sub.} + (R_p - \delta_{tip})\cos\varphi + (R_p - \delta_{sub}) + H\cos\theta \tag{12}$$

In these equations, y_P and z_P are the horizontal and vertical deformations of AFM cantilever, $y_{sub.}$ and $z_{sub.}$ are the horizontal and vertical position of substrate, R_p and R_t the particle radius and tip radius, H is the AFM tip height. Angles θ and φ, respectively, represent the torsion angle of cantilever and pushing

force angle. The values of δ_{sub} and δ_{tip}, which represent the deformations between particle-substrate and particle- AFM tip, respectively, are obtained from the deformation equation of JKR contact theory (i.e., Eq. 11) by respectively substituting the contact radiuses (a) obtained from the combined equations of (14) and (15) (which have been developed from the equations of JKR theory for the contact between cylindrical geometry and flat surface) and Eq. 16 of the JKR theory (which is the equation of JKR theory for the contact between two spheres). Also, ω in Eq. 13 represents the work of adhesion in the biological medium, and it is substituted into Eq. 13, according to the explanations given above. Also, K in Eq. 13 is the equivalent modulus of elasticity of the materials in contact.

$$\delta = \frac{a^2}{R} - \frac{2}{3}\sqrt{3\pi\omega a / K} \tag{13}$$

$$a^{2D}_{JKR} = \left(\frac{2\omega(R_P)^2}{\pi K}\right)^{\frac{1}{3}} \tag{14}$$

$$P^{2D}_{JKR} = 3\left(\frac{\pi k\omega^2 R_P}{16}\right)^{\frac{1}{3}} \tag{15}$$

$$a^3 = \frac{(R_p + R_t)}{K}(P + 3\pi(R_p + R_t)\omega + \sqrt{6\pi(R_p + R_t)\omega P(3\pi(R_p + R_t)\omega)^2}) \tag{16}$$

Since in the liquid medium, the external force (P), in addition to including the externally applied dynamic force (F_1), also includes the main intermolecular forces (i.e., electrostatic force (F_{el}), steric force (F_{steric}) and hydration force (F_{Hyd})), we will have:

$$P = F_1 + F_{el} + F_{steric} + F_{Hyd} \tag{17}$$

Thus, using the Newton-Euler equations, the dynamic equations for the movement of the center of mass of AFM tip in the biological liquid medium will be written as Eqs. 18 through 20.

$$P_Y = F_y + \frac{m}{2}\left(H\dot{\theta}^2\sin\theta\right) \tag{18}$$

$$P_Z = F_z - \frac{m}{2}\left(H\dot{\theta}^2\cos\theta\right) \tag{19}$$

$$M = P_Z H \sin\theta + P_Y H \cos\theta - I\ddot{\theta} \quad (20)$$

Finally, the force exerted by the AFM tip on particle is derived from the following equation:

$$P_T = \sqrt{P_Y^2 + P_Z{}^2} \quad (21)$$

In the above equations, P_Y and P_Z are the components of the force applied on particle by the probe and vice versa and M is torsional torque of cantilever. Figure 2 illustrates the free body diagram of the forces applied on AFM tip and cylindrical nanoparticle during the manipulation process in biological liquid medium as well as some important parameters used in the kinematic and dynamic equations of the process.

The critical conditions in the pushing of particle by the AFM probe occur when the force of the AFM tip overcomes the adhesion and friction forces of the particle and the particle starts to move on the substrate. During the pushing process, the particle is likely to either roll or slide, depending on the contact and frictional conditions.

Sliding of particle on substrate:

$$F_T > \frac{\tau_s A_s}{\sin\psi - \mu_s \cos\psi} = F_{cr_s}^* \quad (22)$$

Rolling of particle on substrate:

$$F_T > \frac{\tau_{r_s} A_s + \tau_{r_t} A_t}{R(\sin\psi + \cos\zeta) + \mu_{r_t} \sin\zeta - \mu_{r_s} \cos\psi}$$
$$= F_{cr_r}^* \quad (23)$$

In view of the above equations, the critical force at the onset of particle movement (F_{cr}) is equivalent to the magnitude of F_T at the moment of overcoming the critical force of $F_{cr_s}^*$ or $F_{cr_r}^*$ and it is a function of ψ (angle of the particle pushing force), φ (probe-particle contact angle), μ and τ (frictional constants), and A_t and A_s (areas of contact between AFM tip-particle and particle-substrate, respectively). The onset of particle movement at each dynamic mode will depend on the establishment of an inequality related to that mode.

Figure 3 shows the algorithm for the dynamic modeling of AFM-based biomanipulation of cylindrical biological particles in various biological mediums.

2.2. Sensitivity analysis method

A quantitative knowledge of a model's sensitivity to the changes of its parameters is a prerequisite for the functional use of the model. To attain this knowledge, Sensitivity Analysis (SA) methods could be employed. Sensitivity analysis is the study of "how the uncertainty in model output (numerical or non-numerical) can be classified into different sources of uncertainty in the input factors of model [24]. A field of sensitivity analysis methods that has attracted more attention is the field of variance-based methods, and the method of Sobol (Sobol, 1993) is considered an efficient approach in this arena. This method is model-independent and it can be applied for nonlinear and non-uniform functions and models. In this method, the sensitivity index is calculated as the share of each input parameter in the overall output variance of the model. The Sobol technique is implemented in four steps: (1) defining the inputs and the type of distribution of each input, (2) generating the samples for the input values, (3) calculating the model output for each set of input samples and (4) determining the effect of each input factor on the output (Tong, 2010). Korayem et al. have been conducted a sensitivity analysis for nano-manipulation of nanoparticles toward dimensional and environmental parameters based on Coulomb and Hurtado and Kim (HK) friction models using Sobol method [15].Korayem et al. have been conducted indentation of three prostate cancer cells CL-1, CL-2 and LNCaP which have lowelasticity modulus and are considered ductile materials using Hertz contact mechanicsmodel [16].
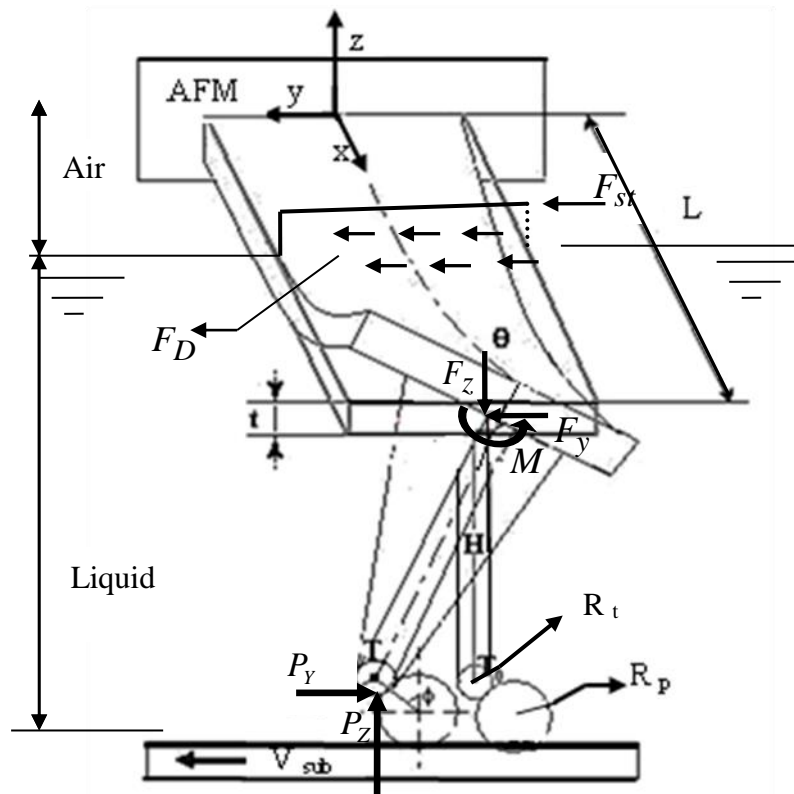
Figure 2. *Free body diagram of the forces applied on the AFM tip and the nanoparticle during the process of pushing \nanoparticles by AFM tip in liquid medium*

Korayem et al. also have been studied the effect of geometry and material of the Micro/Nano particle on contact mechanic for manipulation based on atomic force microscopy [17].

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For a model defined by function $Y = f(X)$, where Y is the model output and $X(x_1, x_2, ..., x_n)$ is the vector of input parameters, Sobol proposed the resolving

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Start

$$y_{p_0} = y_{T_0} = 0, \varphi_0 = 0.7°, z_{p_0} = L\sin\varphi_0$$
$$\dot{y}_{sub} = 100(nm/s), \dot{\theta}_0 = \dot{y}_{sub}/H, \ddot{\delta}_t = \ddot{\delta}_s = 0$$
$$\ddot{y}_{p_0} = \ddot{y}_{T0} = 0, F_{Z_0} = F_{z_0} = K_Z z_{p_0}$$

$$y_{sub} = y_{0sub} + v_{sub}t$$
$$y_p = y_{sub} + (R_p + R_t - \delta_t)\sin\varphi - H\sin\theta$$
$$z_p = z_{sub} + (R_p + R_t - \delta_t)\cos\varphi + (R_p - \delta_s) + H\cos\theta$$

$$\ddot{y}_p = \ddot{y}_{sub} - \ddot{\delta}_t \sin\varphi - H\ddot{\theta}\cos\theta + H\dot{\theta}^2 \sin\theta$$
$$\ddot{z}_p = \ddot{z}_{sub} - \ddot{\delta}_t \cos\varphi - \ddot{\delta}_s - H\ddot{\theta}\sin\theta - H\dot{\theta}^2\cos\theta$$

$$F_Z = F_z - \frac{m}{2}(H\dot{\theta}^2 \cos\theta - H\ddot{\theta}\sin\theta), F_z = K_z z_p$$
$$F_Y = F_y + \frac{m}{2}(H\dot{\theta}^2 \sin\theta - H\ddot{\theta}\cos\theta), F_y = K_y y_p$$
$$M_\theta = F_Z H\sin\theta + F_Y H\cos\theta - I\ddot{\theta}, M_\theta = K_\theta \theta$$
$$F_T = \sqrt{F_Z^2 + F_Y^2}, \psi = \tan^{-1}(F_Y/F_Z)$$

δ_t, δ_s

$$P = F_1 + F_{el} + F_{steric} + F_{Hyd}$$

Contact radius for particle-substrate contact:

$$a_{JKR}^{2D} = (\frac{2\omega R_p^2}{\pi K})^{\frac{1}{3}}, P_{JKR}^{2D} = 3(\frac{\pi k\omega^2 R_P}{16})^{\frac{1}{3}}$$

Contact radius for particle-AFM tip contact:

$$a^3 = \frac{(R_p + R_t)}{K}(P + 3\pi(R_p + R_t)\omega + \sqrt{6\pi(R_p + R_t)\omega P(3\pi(R_p + R_t)\omega)^2})$$

$$F_s^* = \frac{\tau_s A_s}{\sin\psi - \mu_s \cos\psi}$$
$$F_r^* = \frac{\tau_{r_s} A_s + \tau_{r_t} A_t}{(R_p + r)(\sin\psi + \cos\xi) + \mu_{r_t}\sin\xi - \mu_{r_s}\cos\psi}$$

Condition for the sliding $F_T > F_s^*$
Condition for the rolling $F_T > F_r^*$
of particle on substrate

$$t = t_{cr}, F_T = F_{cr}$$
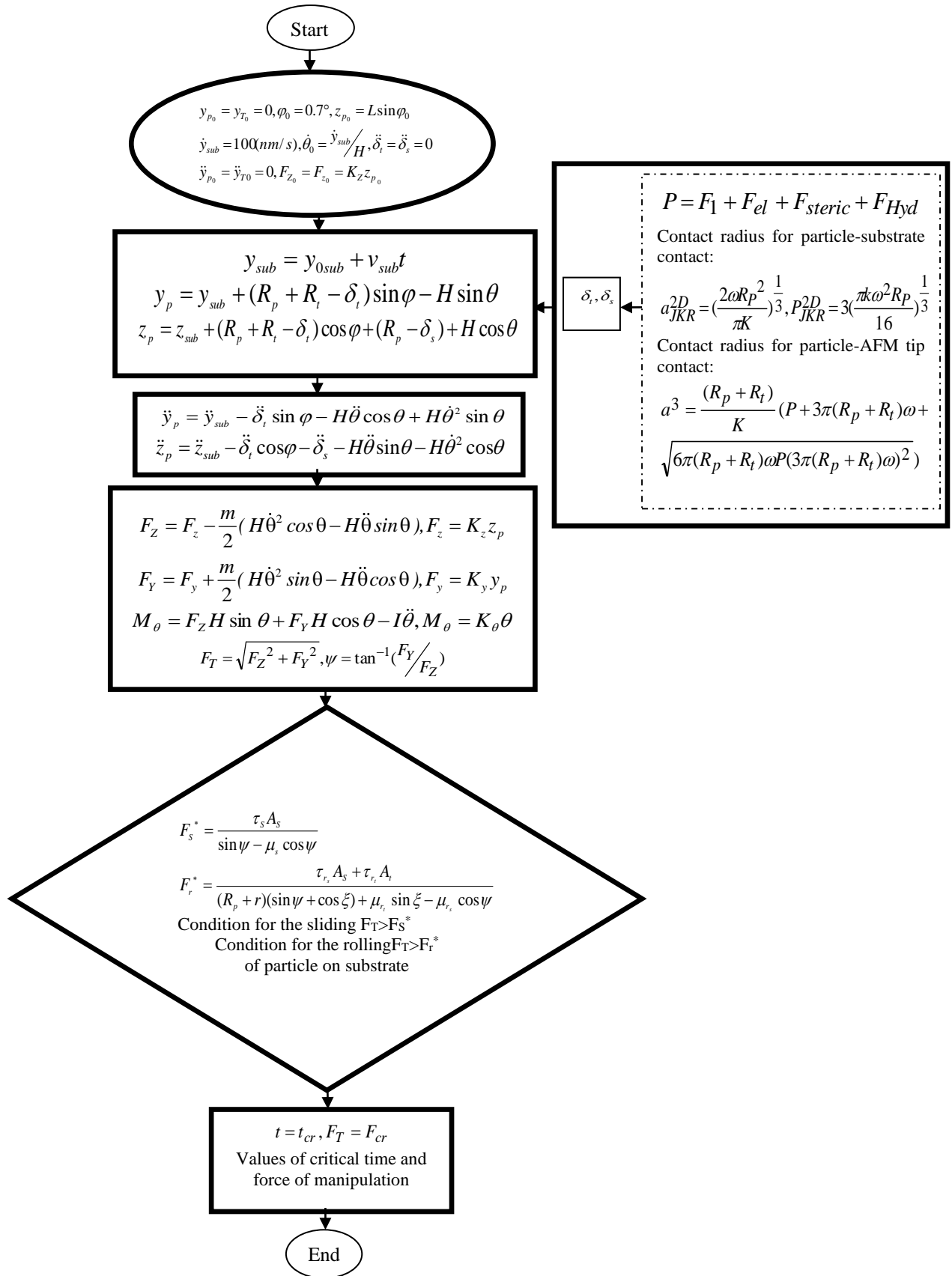Values of critical time and force of manipulation

End

Figure 3. *Algorithm for the dynamic modeling of AFM-based biomanipulation of cylindrical biological particles in various biological mediums.*

of Function Y as summands of increasing dimensionality [21]:

$$f(x_1, x_2, ..., x_n) = f_0 + \sum_{i=1}^{n} f_i(x_i) + \sum_{1 \leq i \leq j \leq n} f_{ij}(x_i, x_j) \quad (24)$$
$$+ \cdots + f_{1,2,...,n}(x_1, x_2, ..., x_n)$$

Where the integral of each term over its input variables is zero. Sobol showed that, when all the inputs are orthogonal to one another, this resolution is unique and the variance of Function Y is the sum of the variances of each resolved term:

$$V(Y) = \sum_{i=1}^{n} V_i + \sum_{i \leq j \leq n}^{n} V_{ij} + ... + V_{1...n} \quad (25)$$

Where

$$V_i = V_{X_i}(E_{X_{\sim i}}(Y|X_i)), \quad (26)$$

$$V_{ij} = V_{X_i X_j}$$
$$(E_{X_{\sim ij}}(Y|X_i, X_j)) - V_{X_i}(E_{X_{\sim i}}(Y|X_i)) - V(E_{X_{\sim j}}(V|X_j)) \quad (27)$$

And this procedure can be repeated for higher-order terms. By dividing Eq. 2 into $Y(V)$, the following equation is obtained:

$$\sum_i S_i + \sum_i \sum_{j > i} S_{ij} + \cdots + S_{12...k} = 1 \quad (28)$$

Where S_i, S_{ij} etc. are called the first-order to higher-order sensitivity indexes. Since the decomposition of variance produces $2^k - 1$ terms, only two indexes are usually computed for each parameter: the first-order sensitivity index (S_i) and the total-order sensitivity index (S_{Ti}), where the tLotal-order index is defined as:

$$S_{Ti} = \frac{E_{X_{\sim i}}(V_{X_i}(Y|X_{\sim i}))}{V(Y)} = 1$$
$$- \frac{V_{X_{\sim i}}(E_{X_i}(Y|X_{\sim i}))}{V(Y)} \quad (29)$$

3. SIMULATION AND RESULTS

In order to automate the manipulation process, it is essential to find out how, and by how much, different process parameters influence the critical values of the manipulation. In this section, the parameters of the manipulation model, in two groups of AFM and environmental parameters have been explored and simulated.

The initial conditions used in simulations are as follows [12]:

$$\begin{cases} \phi_0 = 0.7 \\ z_{p0} = L\sin(\phi_0) \\ z_{T0} = z_{p0} - H \\ \ddot{z}_{p0} = \dfrac{V^2_{sub}}{H} \end{cases} \quad \begin{cases} y_{p0} = y_{T0} = 0 \\ \ddot{y}_{p0} = \ddot{y}_{T0} = 0 \end{cases}$$

$$\begin{cases} \dot{\delta}_{sub} = \dot{\delta}_{sub} = cte \\ \ddot{\theta} = 0 \end{cases} \quad \begin{cases} \theta_0 = 0 \\ \dot{\theta} = \dfrac{V_{sub}}{H} \end{cases}$$

The general procedure of SA is outlined in the flowchart of Figure 4.

3.1. Sensitivity analysis results of AFM parameters

Cantilever-related dimensional parameters such as length, width and thickness are included in the group of AFM parameters. Since in the AFM-based manipulation, cantilever is the part of the AFM probe which plays the key role in the manipulation process and forms the moving part of the instrument in the manipulation environment, it becomes necessary to investigate the effect of its dimensional parameters on the critical values of manipulation.

The changes of the critical manipulation force with cantilever thickness have been shown in Fig. 5(a). According to this diagram, with the increase of this parameter, the critical force for the onset of movement also increases with a sharp slope. Thus, in the group of AFM parameters, the cantilever thickness parameter has a high sensitivity for the critical manipulation force; and since the reduction of cantilever thickness causes the reduction of the critical force of manipulation and the drag force applied on AFM probe on the one hand, and on the other hand, with regards to the stiffness equations in [18], leads to the reduction of

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cantilever stiffness, the selection of an optimal value for this parameter becomes highly important.

Start

Choosing the parameters

Defining the range of changes of

Generating the sample

$i = 1$

$X_i = \begin{bmatrix} x_1^i & x_2^i & \cdots & x_N^i \end{bmatrix}$

The dynamic model for the manipulation of cylindrical nanoparticle

$F_{cr} = F_{cr}(i)$
$i = i + 1$

$i < N_{sim}$

Yes

No

$\begin{bmatrix} F_{cr} \end{bmatrix}_{N_{sim} \times 1}$

Sensitivity analysis

$ST = \begin{bmatrix} ST_1 & ST_2 & \cdots & ST_n \end{bmatrix}$

End
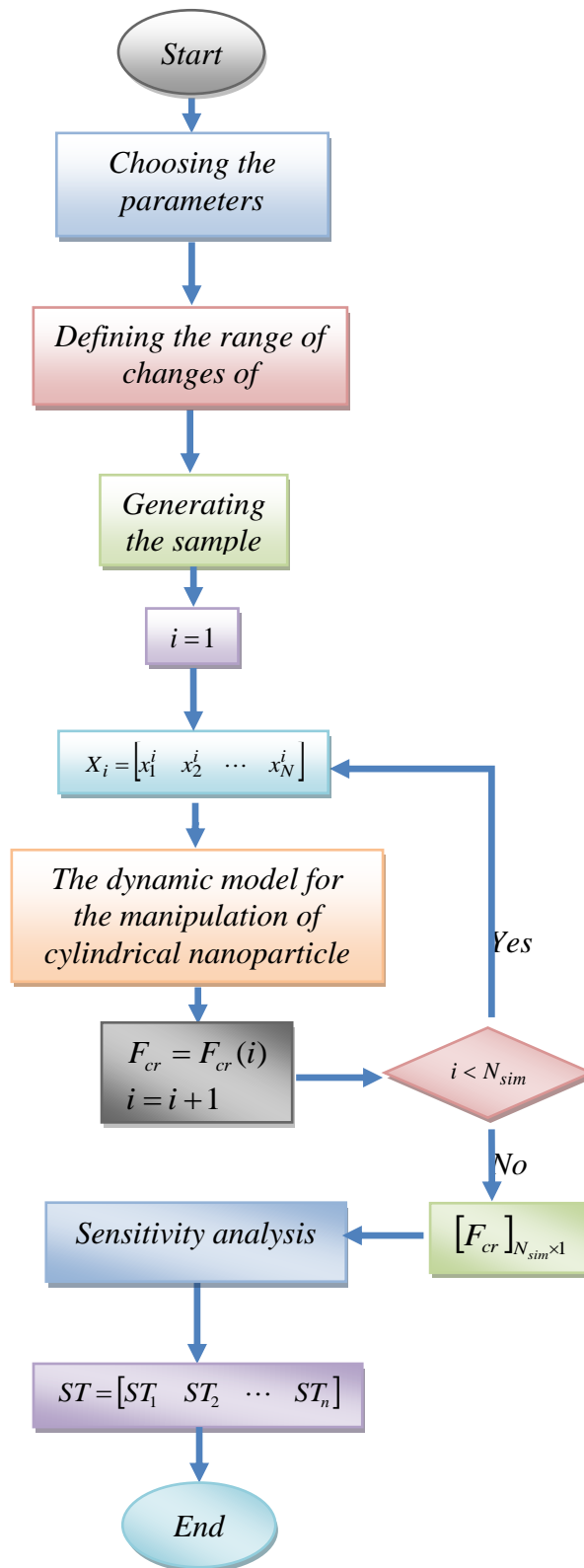
Figure 4. *Algorithm of the sensitivity analysis procedures.*

Fig. 5(b) illustrates the changes of the critical manipulation force with cantilever length. It indicates that, with the increase of this sensitive parameter, the critical force of manipulation diminishes. As is observed in this figure, at low values of cantilever length, sensitivity is greater and with the increase of length, the slope of the diagram becomes milder. So, by considering the results that indicate the effect of this parameter on the critical force of movement, the proper values for this parameter can be selected.

Another sensitive parameter among the AFM parameters is the cantilever width. According to Fig. 5(c), with the increase of this parameter, the critical force for the onset of movement increases with a linear slope. This linear increase indicates that the sensitivity of this parameter is the same in all its range of changes. The diagram of the critical force of manipulation vs. modulus of elasticity (Fig. 5(d)) has been obtained with a positive slope and close to zero. It shows little change in the critical force of movement with the change of elasticity modulus; and so, this is not considered a sensitive parameter in the manipulation process. Therefore, in the manipulation of nanoparticles, there is no restriction in selecting the cantilever material; and depending on the conditions of the manipulation environment, the proper material can be selected for the cantilever. The other AFM parameter is the height parameter; and considering a near zero slope for the diagram showing the changes of the critical manipulation force vs. cantilever height (Fig. 5(e)), this parameter is not considered to be a sensitive parameter either for the critical force of manipulation, and choosing different values for this parameter from its range of changes doesn't lead to a tangible change in the manipulation values. The last AFM parameter which has been examined is submerged length of the cantilever. The diagram of the critical force of movement

versus submerged length of the cantilever has been shown in Fig. 5(f) with a positive, and near zero, slope. With the change of submerged length of the cantilever in its range of variations, a minor change is observed in the critical manipulation force, and so this parameter is not considered as a sensitive parameter for the critical force of manipulation.

3.2. Sensitivity analysis of environmental parameters

In this section, the environmental parameters that influence the manipulation process are explored. Fig. 6 shows the changes of the critical force for the onset of movement versus the environmental parameters.

In view of Figs. 6(a) through 6(c), it is observed that the diagrams of the changes of the critical force of movement versus the parameters of cross sectional radius of particle, the α parameter (In this study, the α parameter is defined as the ratio of the length of a cylinder to its cross sectional radius) and the work of adhesion in the biological environment, respectively display steeper slopes. Therefore, among the environmental parameters that affect the critical force and time of manipulation, the dimensional parameters of the target particle are the most sensitive, followed by the work of adhesion in the biological environment. The most sensitive dimensional parameter for the critical force of movement of the target cylindrical micro/nanoparticle is the cross sectional radius of particle. As is shown in Fig. 6(a), with the increase in the cross sectional radius of particle, the critical manipulation force also increases with a very sharp slope. The second most sensitive parameter is the dimensional parameter (α), which was defined as the ratio of the length of the target cylindrical particle to its cross sectional radius. According to Fig. 6(b), with the increase of this parameter, the

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critical force of manipulation increases considerably.

The third most sensitive environmental parameter to which the critical force of movement is sensitive is the work of adhesion in the biological environment; and with the increase of this parameter, the critical force for the onset of particle movement increases as well (Fig. 6(c)). This is something expected, since with the increase in the amount of the work of adhesion in the biological environment, the pull-off force necessary in the manipulation process also increases.

As Figs. 6(d) through 6(g) demonstrate, the diagrams showing the changes of the critical manipulation force versus the other investigated parameters in the simulation, including the elasticity modulus of particle, Poisson's ratio, substrate velocity and the viscosity of the biological fluid selected as the manipulation medium, have very mild and near zero slopes. This indicates that by altering the values of these parameters in their respective ranges, no substantial change will be induced in the critical force of manipulation. In investigating the effect of the organic micro/nanoparticles' modulus of elasticity, a range of 75 to 125 (MPa) has been covered in the simulations, which includes a wide spectrum of biological particles such as platelets (which have spherical or disk-shaped bodies with a diameter of 2-4 μm), yeasts (which have spherical or elliptical forms or a geometrical shape between sphere and cylinder (like a circular-crowned cylinder)), human cells, DNA and many bacteria and viruses.
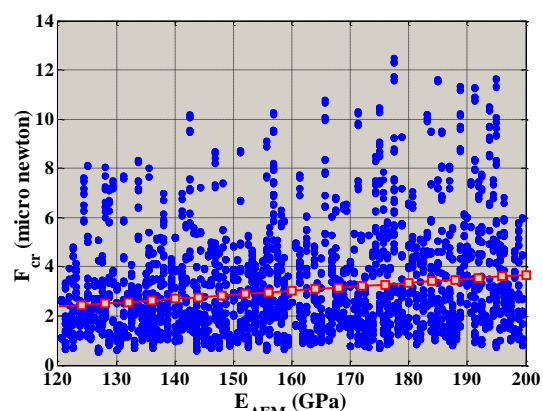

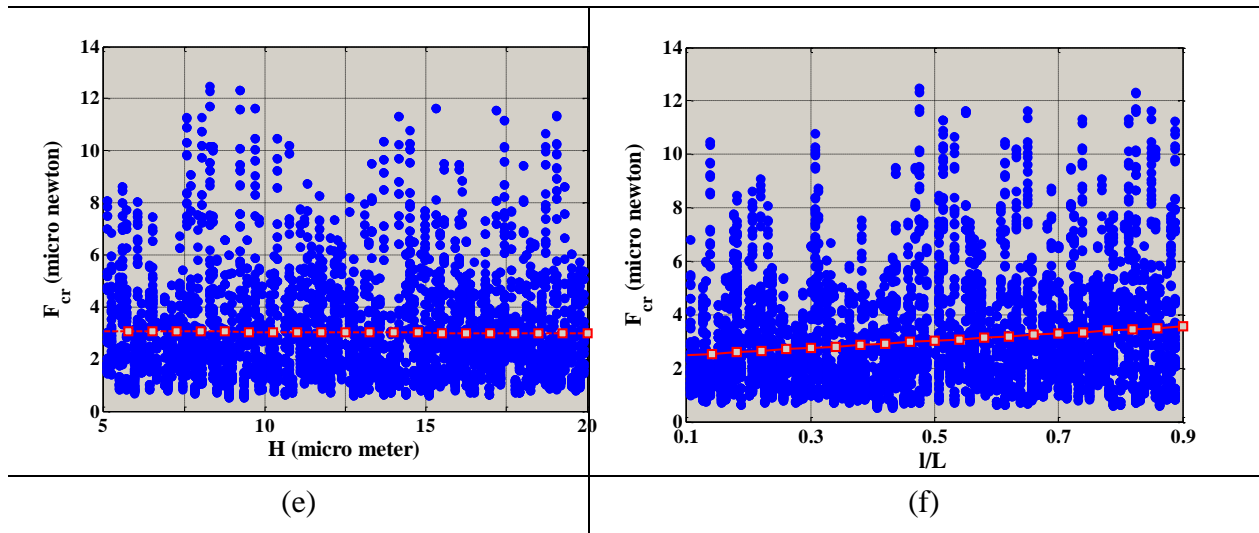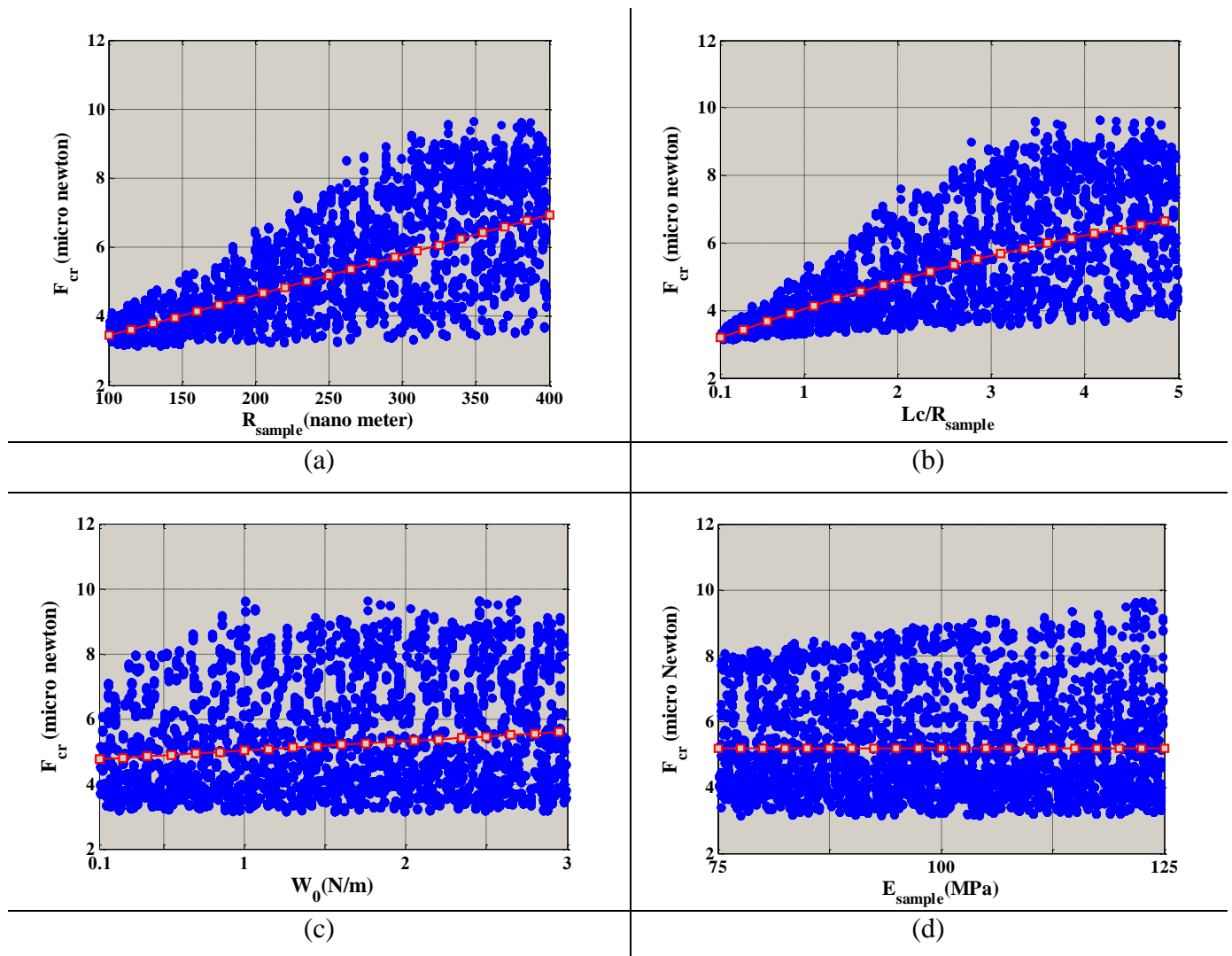
(a)

(b)

(c)

(d)

(e)

(f)
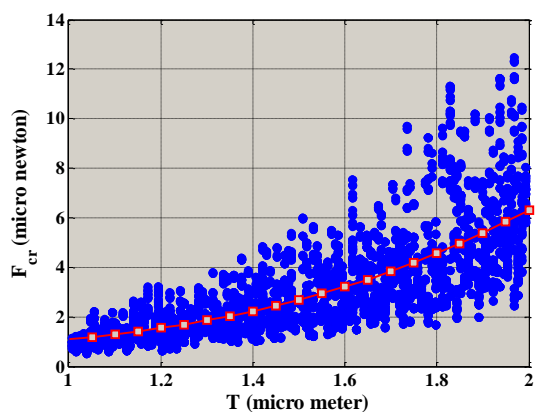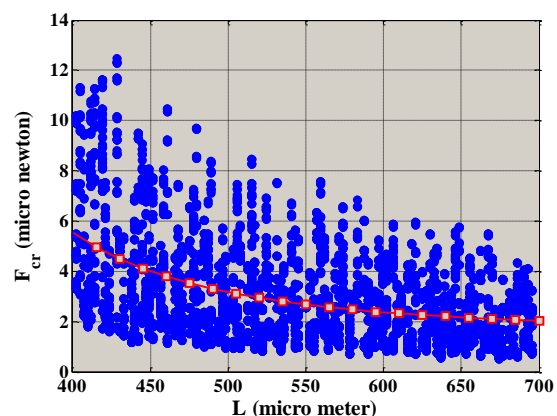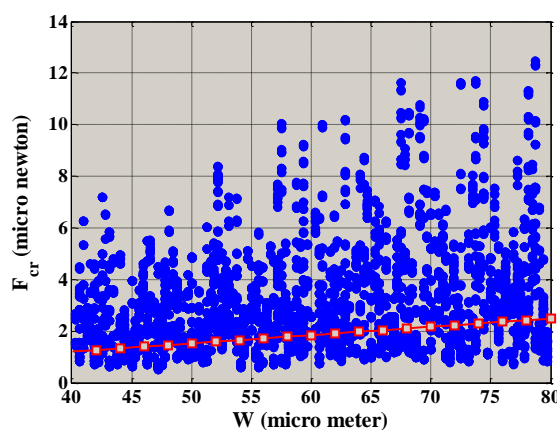
Figure 5. Sensitivity of the critical force of movement to AFM parameters in the manipulation of cylindrical nanoparticles in biological environments.
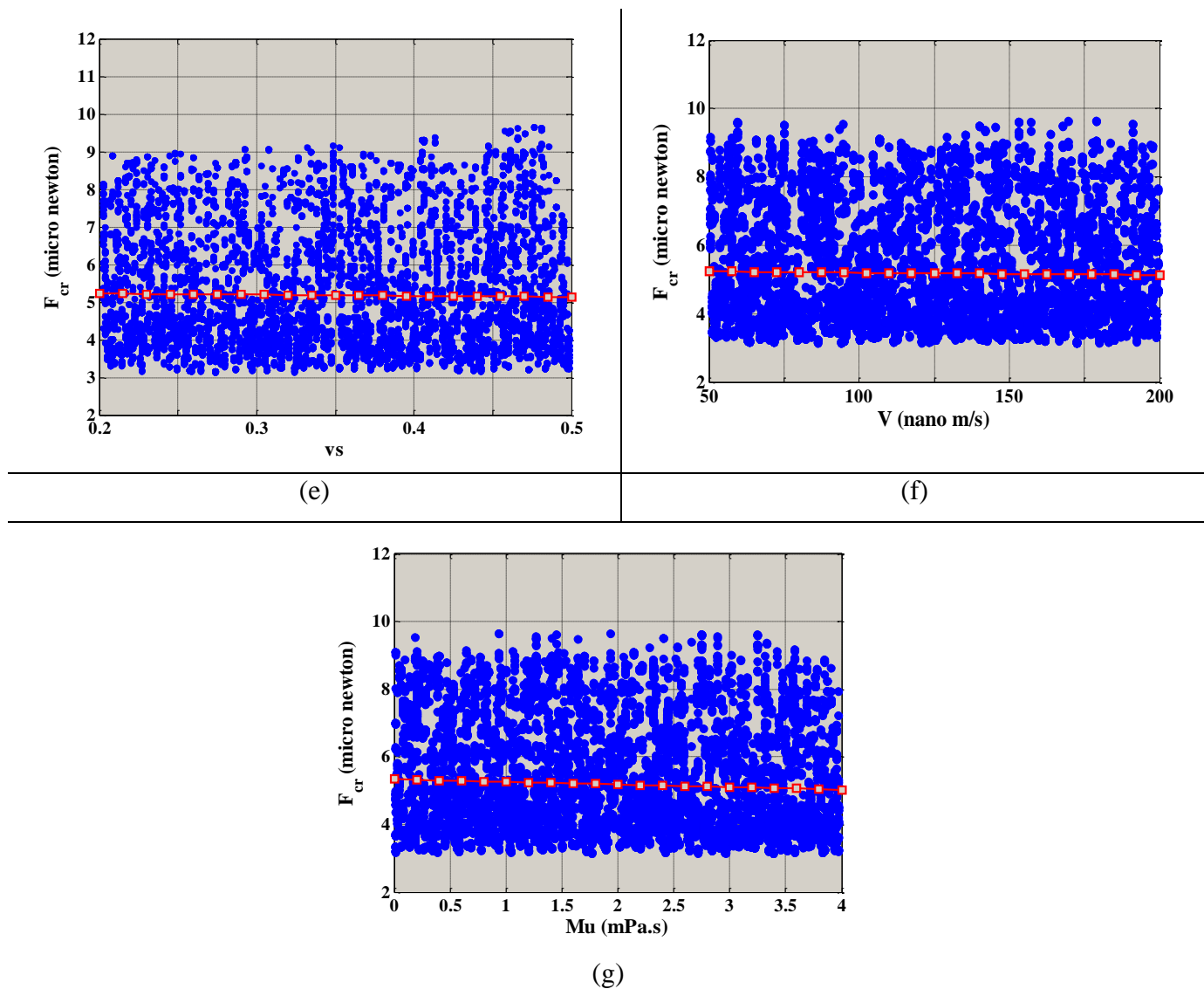

(a)

(b)

(c)

(d)

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Figure 6. *Sensitivity of the critical force of movement to environmental parameters in the manipulation of cylindrical nanoparticles in biological environments.*
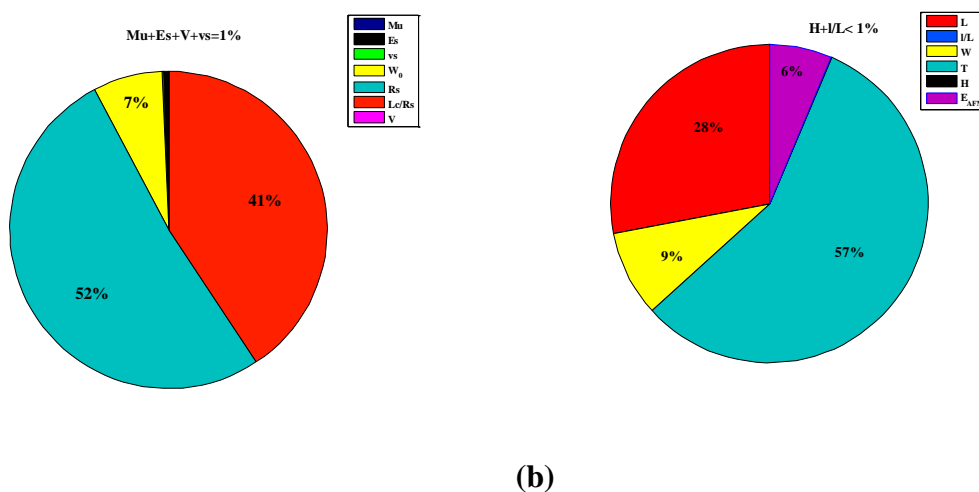

(a)   **(b)**

Figure 7. *(a) Percent sensitivity of the critical force of movement to environmental parameters; (b) Percent sensitivity of the critical force of movement to AFM parameters.*

Fig.7. indicates more accurate analysis of the results obtained by the Sobol sensitivity analysis method for the parameters of the dynamic model of cylindrical micro/nanoparticles manipulatio in biological environments.

Fig. 7 demonstrates that the dimensional parameters are the most important ones both in AFM parameters group and in environmental parameters group.

According to this figure, As expected, cross sectional radius of particle (with a sensitivity index of 52%), the α parameter (with a sensitivity index of 41%) and the work of adhesion in the biological environment (with a sensitivity index of 7%), are of most significant sensitivity among 7 environmental parameters and in AFM parameters group (Fig. 5(b)), cantilever thickness (with 57% sensitivity) is the most important parameter, and the parameters of cantilever length and width (with 28% and 9% sensitivities, respectively) are the other effective AFM parameters.

4. VERIFICATION

To validate results obtained in this study, the results of simulations for dimensional parameters are compared with the existing results in previous research works [13].
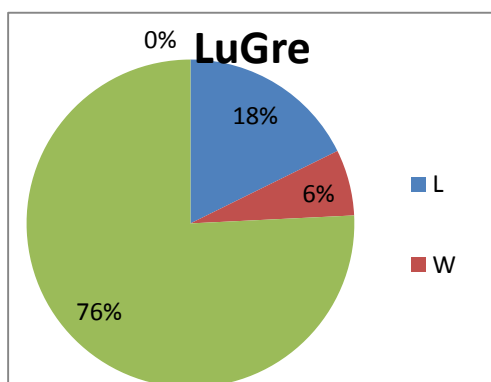

Figure 8. *Existing results for dimensional sensitivity analaysis [13].*

Comparison between Figure 7(b) and Figure 8 indicates that in this study same as previous researches, among all

dimensional parameters, the cantilever width and the Cantilever length parameters are the most and the second most sensitive parameters, respectively. Hence, the comparison results demonstrate that the results of this study are verified with existing results of previous researches.

5. CONCLUSION

The manipulation of micro/nanoparticles is a complex process, and there are still many unknowns about the way this process is influenced by various parameters. In this paper, the sensitivities of the dynamic model for the manipulation of biological nanoparticles to different parameters that influence the manipulation process were investigated for two groups of AFM and environmental parameters, and the sensitivity of each parameter to the critical force of manipulation was determined.

In the group of AFM parameters, the three parameters of cantilever thickness, length and width showed a higher sensitivity relative to the parameters of AFM modulus of elasticity and probe height. Cantilever thickness, with a sensitivity of 50%, is the most sensitive AFM parameter. The reduction of cantilever thickness leads to the reduction of the critical manipulation force as well as the reduction of the drag force exerted on cantilever in biological liquid mediums, which are favorable achievements in the implementation of the manipulation process. On the other hand, the reduction of thickness leads to the reduction of cantilever stiffness, which makes the cantilever more susceptible to damage. Therefore, in selecting the right cantilever thickness, a compromise should be made between the above issues in order to determine the optimal cantilever thickness.

By increasing the cantilever length, the critical manipulation force diminishes. At lower values of this parameter, its sensitivity is higher. Due to the very mild and positive slope of the curve of cantilever width changes, the sensitivity of

this parameter is the same along its range of changes, and so by choosing lower values for this parameter, the critical force needed for the onset of particle movement can be reduced.

In view of the results obtained in the simulations, the cross sectional radius of target particle (with a sensitivity of 52%) is the most sensitive environmental parameter. With the increase of this parameter, the critical force of movement increases considerably. With a sensitivity of 41%, the second rank of sensitivity among the environmental parameters belongs to the α parameter. The range of changes of this parameter in the simulations has been chosen in such a way that a vast range of particles with a basic cylindrical geometry, from rod shaped particles to disk-shaped particles (with an aspect ratio of less than 1.0), is included in the investigation. With the increase in the value of this parameter, the critical manipulation force increases with a steep slope. With the increase in the area of contact between particle and substrate and the increase of the adhesion and friction forces, as the values of these two sensitive dimensional parameters increase, the increase in the magnitude of the critical manipulation force is expected.

The parameter of the work of adhesion in the biological environment, with a sensitivity of 7%, occupies the third rank of sensitivity among the environmental parameters. At lower values of this parameter, a lower critical force of movement is obtained, which is something desired. Based on the calculations of the work of adhesion in the biological environment and simulation results with the surface energy of the target organic particle getting closer to that of the chosen biological medium, a lower work of adhesion in the biological environment is obtained and consequently, the critical manipulation force also becomes less; therefore, this finding could be applied in the selection of a suitable medium for the manipulation of biological particles.

The elasticity modulus parameter doesn't show a significant sensitivity in its range of changes. Due to the negligible sensitivity of this parameter, any variation in the material types of biological particles, in the range considered in simulations, doesn't produce much of a change in the critical force needed for particle movement; and the same conclusion is valid for the Poisson's ratio parameter.

Also, the parameters of substrate velocity and the viscosity of the biological manipulation environment don't exhibit a substantial sensitivity variation in their range of changes and therefore, these two parameters are not considered as significant parameters in the manipulation processes conducted in biological mediums.

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