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Viscoelastic properties of cells: Modeling and identification by atomic force microscopy

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ABSTRACT

Identification of mechanical properties of cells has previously been shown to have a great potential and effectiveness on medical diagnosis. As a result, it has gathered increasing interest of researchers over the recent years. Atomic force microscopy has become one of the prime technologies for obtaining such properties. Traditionally, local variations in elasticity has been obtained by mapping contact force during sample indentation to static Hertzian contact models. More recently, multiharmonic methods have allowed for both viscous and elastic measurements of soft samples. In this article, a new technique is presented based on dynamic modeling and identification of the sample. Essentially, the measured signals are mapped to the sample properties of the model in a least-square sense. This approach allows for easy extensibility beyond pure viscoelastic measurements. Furthermore, an iterative modeling approach can be used to best describe the measured data. The technique can be operated in either dynamic indentation viscoelastic mode, or scanning viscoelastic mode. First, a dynamic, viscoelastic model of the sample is presented. Then, the parameter identification method is described, showing exponential convergence of the parameters. A simulation study demonstrates the effectiveness of the approach in both modes of operation.

1. Introduction

Since its invention in 1986, atomic force microscopy (AFM) has become one of the leading technologies for imaging sample surfaces at nanometer scale resolutions [1]. In the beginning, AFM was applied almost exclusively to characterize the surfaces of nonbiological materials [2], and even today, its major applications are still in the visualization of microcircuits, material sciences and nanotechnology [3]. However, application of AFM to biological and biomedical research has increased exponentially during the recent years [4], since the AFM enjoys several advantages over conventional optical microscopes and electron microscopes, especially concerning studies of biological samples [1].

The main beneficial feature of AFM in the study of biological samples is its ability to study the objects directly in their natural conditions. Other advantages include: [5] 1) AFM can get information about surfaces in situ and in vitro, if not in vivo, in air, in water, buffers and other ambient media, 2) it has an extremely high scanning resolution, up to nanometer (molecular) resolution, and up to 0.01 nm vertical resolution, 3) it provides true 3D surface topographical information, 4) it can scan with a wide range of forces, starting from virtually zero to large destructive forces, 5) it allows measuring various biophysical properties of materials including elasticity, adhesion, hardness, and friction.

In order to improve early diagnosis of cancer there is an urgent need to increase understanding of cancer biology on a cellular level. Single cell deformability has been studied for a long time using various techniques. The driving force for such studies is the assumption that, depending on disease type, the altered cellular deformability should play a critical role in the development and progression of various diseases [6]. So far, several approaches have been investigated, including methods such as micro-pipette manipulation [7], magnetic bead twisting [8], and optical tweezers [9]. With these techniques, local variations in the viscoelastic power law parameters have been observed [10].

AFM has enjoyed many improvements to its main capabilities from a control engineering point of view [11–18]. Additionally, effort has been put into trying to discover the potential of AFM in cancer detection [1,5,6,10,19–23]. Any research result that would provide the possibility of an early and easy diagnosis of carcinoid cells with accuracy is of extreme interest to specialists that deal with the diagnosis and cure of the disease. In [24] AFM measurements of the human breast biopsies reveal unique mechanical fingerprints that help define the

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stages of cancer progression. High-resolution stiffness mapping shows that in addition to matrix stiffening, tumor progression is due to softening of the tumor cells.

Clearly, mechanical properties of cells are of high interest to the research community. The elastic modulus of biological samples are typically found through force-indentation procedures [25,26], performed statically. That is, by indenting the sample and measuring the deflection against the commanded cantilever position. By fitting the resulting curve to the Hertz contact model, the elastic modulus of the sample can be determined. Furthermore, extensions to the Hertz model allow for determining the adhesion between the tip and sample from the force-distance curve [27–29].

Several variations to the force-indentation approach exist [29,30]. In force-volume imaging, force-indentation curves are gathered at multiple points across the sample, each point allowing for determining elasticity and adhesion. In the PeakForce quantitative nanomechanical method, the cantilever is oscillated below resonance (typically 1kHz) with each oscillation resulting in a force-indentation curve, allowing for improved imaging speeds. Related developments perform frequency-dependent mapping of the nanomechanical properties [31]. Furthermore, some techniques have been proposed for additionally determining viscous properties from force-indentation curves by exploiting the time-history of the data [32,33].

Early results in AFM have shown that the amplitude and phase of a dynamically excited cantilever can be mapped to both elastic and viscous properties of the sample [34]. More recently, multiharmonic approaches have been developed for mapping mechanical properties of cells [35,36]. Here, the cantilever is typically excited at resonance frequency. As the cantilever is scanned across the sample, the measurable signals are mapped to local mechanical properties such as elasticity and viscosity. This approach allows for a considerable increase in acquisition speed.

In this article a new technique for the identification of viscoelastic properties of soft samples in AFM is presented, first developed in [37,38]. Here, the sample is modeled as a dynamic model with unknown parameters, in terms of a laterally spaced grid of spring constants and damping coefficients. The parameters are identified from the measurable signals, using tools from the control literature. Essentially, the measurable signals are mapped to the parameters of the sample model recursively in a least squares sense, making it possible to observe changes over time. The estimated parameters are guaranteed to converge to the real values exponentially fast provided a suitable control input is chosen.

This technique can be operated in two distinct modes. In dynamic indentation viscoelastic (DIVE) mode [37], mechanical properties of the sample are identified at a discrete number of points by indenting in and out of the sample. In the scanning viscoelastic (SVE) mode [38], viscoelastic properties are gathered in a continuous fashion as the sample is scanned along the lateral axes at constant depth. In this article the two modes are expanded upon and compared.

In Section 2 a system model description of the viscoelastic sample is designed, suitable for parameter identification. The two modes of operation, DIVE and SVE, are presented in Section 3. Next, Section 4 presents the identification techniques for the unknown parameters of the system. Results are given in Section 5. In Section 6 the technique is discussed in the context of previous approaches and future considerations, and in Section 7 final conclusions are reached.

2. System modeling

In this section, the system modeling is presented. This includes the dynamics of the sample, the cantilever dynamics, the geometry of the tip and their combinations, in order to acquire a full system description. The purpose of the modeling is to provide a description of the interaction between the cantilever and a general viscoelastic sample material, while allowing for simple identification of the model parameters by the usage of the atomic force microscope.

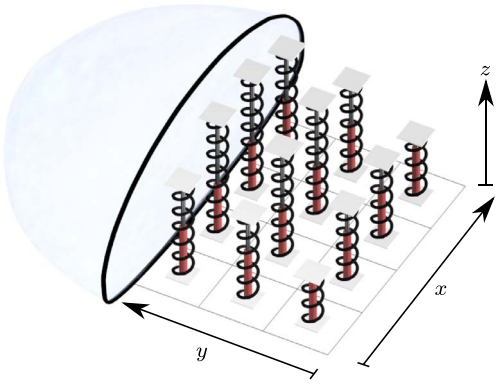

Fig. 1. The sample is modeled as spring-damper elements evenly spaced along the lateral axes.

2.1. Sample dynamics

The modeled sample is considered as a system of discrete spring-damper elements, as illustration in Fig. 1. The elements are evenly distributed in the lateral *xy*-axes, and can be compressed in the vertical *z*-direction [37,38].

The interaction between the AFM cantilever and the sample is analytically presented, and illustrated in Fig. 2. The position of the tip along the *xyz*-axes is denoted by (*X, Y, Z*). The vertical tip position *Z*, the cantilever deflection *D*, and the controllable cantilever base position *U* are related by

$$Z = U - D. \tag{1}$$

Since the deflection *D* is measurable and *U* is controllable, all three signals are assumed to be available.

The dynamics between the cantilever and the sample can be described by three main components as seen in Fig. 3. The cantilever dynamics is subject to an external sample force which generates a deflection along the vertical axis. The tip geometry and position is then used to determine the (possibly compressed) positions of each sample spring-damper element. The compressed elements in turn creates a restoration force acting on the cantilever tip. The details of each of these

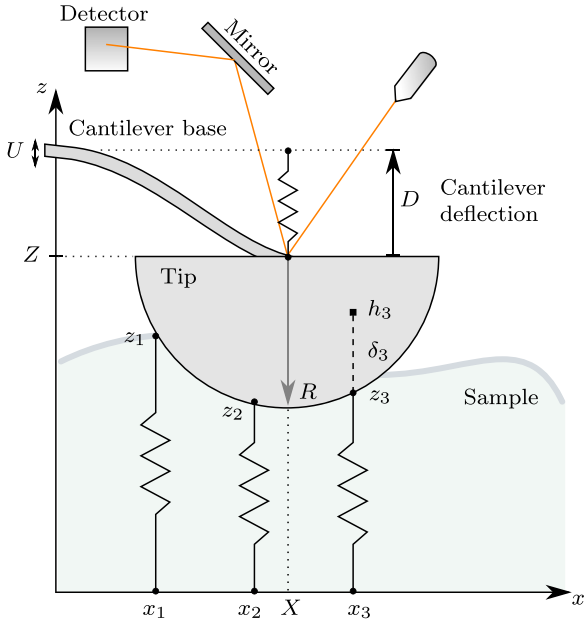

Fig. 2. Indentation of the tip into the sample.