ABSTRACT

Atomic force microscopy (AFM) is a powerful and increasingly common modality of biomechanical investigation, including imaging, force spectroscopy, and microrheology. AFM indentation of biomaterials requires use of a contact model for data interpretation and material property extraction, and a large segment of the scientific community uses the Hertz model or a close relative for small-scale indentation of thin, soft materials in high strain applications. We present experimental results and analytical/numerical modeling which lead to two main conclusions: (i) Hertzian mechanics are useful in a surprisingly large parameter range, including scenarios in which the underlying assumptions are seemingly violated, and (ii) the Hertz solution serves as a useful base from which power-series type solutions can be derived for a variety of non-Hertzian effects.

EXPERIMENTAL APPROACH

We prepared samples of low-concentration agarose gel (typically about 1% w/v) whose modulus should typically be in the 10 – 50 kPa range for indentation testing in the AFM. We prepared both thin ($h \approx 1 \mu m$) and thick ($h \approx 1 \text{ mm}$) gels. A Digital Instruments Nanoscope III was used for the indentation experiments. Soft tipless cantilevers (nominal $k = 0.06 \text{ N/m}$) had spherical glass beads adhered to their end (Novascan, Inc.), with bead radius ranging from 1 – 5 $\mu m$. The gels were mounted on the AFM stage and the cantilever was mounted in a fluid cell. The assembled system remained undisturbed for about 30 minutes so that thermal equilibrium could be achieved. The experiment then proceeded in several steps. First a topographic image of the gel surface was collected in contact mode. Second, specific points on the surface were indented and force curves were collected. The indentation depth varied over the range $1 < \delta < 200 \text{ nm}$, with a corresponding indentation force ranging up to about 10 nN. At least three indentations were taken at each location, and the force curves were then averaged.

MATHEMATICAL MODELING

Figure 1 shows the mathematical model of the indentation. Two key issues make extracting elastic properties from the AFM
raw data challenging: (i) identification of the first contact point, and (ii) presence of nonlinear elastic (hardening) response at deep indentation. Our thin samples also exhibit finite thickness effects, especially for large $\delta/h$, while the thick samples do not. The nominal indentation mechanics can be reasonably described by the Hertz model, and we have developed power-series-type corrections to the Hertz model to account for first contact point misidentification and the deep-indentation hardening response.

The nominal Hertzian relationship between indentation force $F$ and indentation depth $\delta$ is $F = A\delta^{1.5}$, where the exponent 1.5 arises naturally from the underlying assumptions of the Hertz model and $A$ is a collection of elastic and geometry parameters. We have augmented the Hertz model with two corrections:

$$F = a_1\delta^{0.5} + A\delta^{1.5} + b_1\delta^{2.5} + \cdots$$  \hspace{1cm} (1)

where only the leading terms of the corrections are shown. The contact point correction is derived through careful consideration of the mechanics of initial touchdown of the cantilever tip on the material surface. The coefficient $a_1$ is primarily a function of the error in contact point identification. The hardening correction is derived from a series of numerical (FEA) experiments, and the leading coefficient $b_1$ is a function of the material description (we have used an exponential material model) and the normalized indentation depth $\tilde{\delta}/R$. The details of both corrections are presented elsewhere [2, 3].

RESULTS

The aqueous environment of the experiments typically masks any adhesion/snap-to-contact events, and therefore identifying the first contact point between the cantilever tip and the sample is often challenging. Moreover, the elastic modulus identified from AFM data is sensitive to the selected first contact. Our power series correction to Hertz reduces this sensitivity by explicitly including contact point misidentification in the parameter identification model. Figure 2 shows raw AFM data (cantilever deflection $d$ as a function of $z$–piezo position) for a thick sample, along with three candidate first contact points. The inset to the figure shows the extracted modulus using the Hertz approach and the contact point corrected model of equation (1). The Hertzian fits to the data are quite sensitive to the contact point selection, while the corrected model shows excellent consistency regardless of the chosen point. In practice, we would typically pick point #3 as our initial contact point because it has a high signal-to-noise ratio and is visually known to be in contact. This approach relieves the requirement for careful contact point selection because the power series correction to the Hertz model fully accounts for any contact point identification errors.

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