

AFM BASED FORCE-SPECTROSCOPY OF SOFT-MATERIALS: POLYMER GELS AND BIOLOGICAL CELLS

VISHWANATH MANAGULI



**DEPARTMENT OF APPLIED MECHANICS
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AFM BASED FORCE-SPECTROSCOPY OF SOFT-MATERIALS: POLYMER GELS AND BIOLOGICAL CELLS

by

Vishwanath Managuli
Department of Applied Mechanics

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to the



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Dedicated to

My Teachers and Family

Who endured all the sufferings silently
and looked forward to this day

CERTIFICATE

This is to certify that the thesis entitled “**AFM Based Force-Spectroscopy of Soft-Materials: Polymer Gels and Biological Cells**” being submitted by **Mr. Vishwanath Managuli** to the Indian Institute of Technology Delhi for the award of degree of **Doctor of Philosophy** in Applied Mechanics is a record of original, bonafide research work carried out by him under my supervision and guidance. The thesis work, in my opinion, has reached the requisite standard fulfilling the requirements for the degree of Doctor of Philosophy.

The Results contained in this thesis have not been submitted in full or in part, to any other university or institute for the award of any degree or diploma.

Date:

Place: New Delhi

Dr. Sitikantha Roy
Assistant Professor
Department of Applied Mechanics
Indian Institute of Technology Delhi
New Delhi-110016, India

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ABSTRACT

The atomic force microscopy (AFM) based force spectroscopy is a nano-mechanical technique used in imaging soft matters, measuring surface forces, manipulating surface features and extracting local mechanical properties at the nano-scale through nanoindentation. The local mechanical properties can be elasticity, adhesion, and viscoelastic properties. Depending on the length scale of operation, force spectroscopy can be categorized as single molecule force spectroscopy, cellular scale force spectroscopy or spectroscopy at bulk material scale, example like thin film analysis, etc. Whatever be the length scale of operation, AFM force spectroscopy needs a contact model to analyze the interaction between the AFM probe and the sample material, and a set of robust protocols for experimental data extraction and analysis.

Present research work is focused on the development of contact models and experimental methods for AFM force spectroscopy technique in soft material characterization, mainly polymer gels and live biological cells. We have addressed two of the most common issues in the analysis of nanoindentation data of soft materials. Firstly, the correction to bottom substrate effect arising during thin sample studies and second, the contact model for simultaneous evaluation of nonspecific adhesion property along with elasticity. The developed contact model has applications in the characterization of soft materials showing adhesive elastic nature. The effectiveness of improvements made in the contact models is experimentally validated by testing transversely isotropic polymer gels of different stiffness and live MCF-7 adenocarcinoma cancer cells. Further, the developed correction factor for finite thickness correction is incorporated into a dynamic contact model for the study of viscoelastic nature of live cells. The model is applied in the study of micro-rheology of Human Mesenchymal stem cells and HCT-116 Colorectal cancer

cells. The main advantage of the proposed model is its closed-form expression, making it easy to use for AFM force spectroscopic data analysis.

AFM-based force spectroscopy technique and incorporated corrections to contact models have great significance in the biomechanical approach of studying diseases and drug effects, but their utilization in the evaluation of biomechanical properties needs a thorough understanding of uncertainties influencing the study. The uncertainties arising from sample preparation, errors from AFM instrument and experimental parameters, the limitations of contact models are discussed along with possible solutions to them. Eventually, it will help in the error-free evaluation of nanomechanical properties of any unknown soft samples.

परमाणु बल माइक्रोस्कोपी (एएफएम) आधारित बल स्पेक्ट्रोस्कोपी एक नैनो-मैकेनिकल तकनीक है जो नरम मामलों को इमेजिंग, सतह की ताकत को मापने, सतह की सुविधाओं में हेरफेर करने और नैनो-स्केल पर नैनो-स्केल पर स्थानीय यांत्रिक गुणों को निकालने में उपयोग की जाती है। स्थानीय यांत्रिक गुण लोच, आसंजन, और viscoelastic गुण हो सकता है। ऑपरेशन के लम्बाई पैमाने पर निर्भर करते हुए, बल स्पेक्ट्रोस्कोपी को एकल अणु बल स्पेक्ट्रोस्कोपी, सेलुलर स्केल फोर्स स्पेक्ट्रोस्कोपी या स्पेक्ट्रोस्कोपी के रूप में वर्गीकृत किया जा सकता है, थोक सामग्री पैमाने पर उदाहरण, पतली फिल्म विश्लेषण आदि जैसे उदाहरण। ऑपरेशन के लम्बे पैमाने पर जो कुछ भी हो, एएफएम बल स्पेक्ट्रोस्कोपी जरूरतों एएफएम जांच और नमूना सामग्री के बीच बातचीत का विश्लेषण करने के लिए एक संपर्क मॉडल, और प्रयोगात्मक डेटा निष्कर्षण और विश्लेषण के लिए मजबूत प्रोटोकॉल का एक सेट।

वर्तमान शोध कार्य नरम सामग्री विशेषता, मुख्य रूप से बहुलक जैल और जीवित जैविक कोशिकाओं में एएफएम बल स्पेक्ट्रोस्कोपी तकनीक के लिए संपर्क मॉडल और प्रयोगात्मक तरीकों के विकास पर केंद्रित है। हमने नरम सामग्रियों के नैनोइंडेंटेशन डेटा के विश्लेषण में दो सबसे आम मुद्दों को संबोधित किया है। सबसे पहले, पतले नमूना अध्ययन और दूसरे के दौरान उत्पन्न होने वाले नीचे सबस्ट्रेट प्रभाव में सुधार, लोच के साथ गैर-विशिष्ट चिपकने वाली संपत्ति के साथ-साथ मूल्यांकन के लिए संपर्क मॉडल। विकसित संपर्क मॉडल में चिपकने वाला लोचदार प्रकृति दिखाते हुए नरम सामग्री की विशेषता में अनुप्रयोग हैं। संपर्क मॉडल में किए गए सुधारों की प्रभावशीलता को विभिन्न कठोरता के ट्रांसवर्सली आइसोटोपिक पॉलिमर जैल और एमसीएफ -7 एडेनोकार्सिनोमा कैंसर कोशिकाओं के लाइव परीक्षण द्वारा प्रयोगात्मक रूप से मान्य किया जाता है। इसके अलावा, सीमित मोटाई सुधार के लिए विकसित सुधार कारक जीवित कोशिकाओं की viscoelastic प्रकृति के

अध्ययन के लिए एक गतिशील संपर्क मॉडल में शामिल किया गया है। मॉडल मानव Mesenchymal स्टेम कोशिकाओं और एचसीटी-116 कोलोरेक्टल कैंसर कोशिकाओं के सूक्ष्म-विज्ञान के अध्ययन में लागू किया जाता है। प्रस्तावित मॉडल का मुख्य लाभ इसकी बंद-रूप अभिव्यक्ति है, जिससे एएफएम बल स्पेक्ट्रोस्कोपिक डेटा विश्लेषण के लिए उपयोग करना आसान हो जाता है।

एएफएम आधारित बल स्पेक्ट्रोस्कोपी तकनीक और मॉडलों से संपर्क करने के लिए शामिल सुधारों में बीमारियों और नशीली दवाओं के प्रभावों का अध्ययन करने के बायोमेकेनिकल दृष्टिकोण में बहुत महत्व है, लेकिन बायोमेकेनिकल गुणों के मूल्यांकन में उनके उपयोग के अध्ययन को प्रभावित करने वाली अनिश्चितताओं की पूरी तरह से समझदारी की आवश्यकता है। नमूना तैयारी से उत्पन्न अनिश्चितता, एएफएम उपकरण और प्रयोगात्मक मानकों से त्रुटियों, संपर्क मॉडल की सीमाओं पर चर्चा के साथ चर्चा की जाती है। आखिरकार, यह किसी भी अज्ञात मुलायम नमूने के नैनोमेकेनिकल गुणों के त्रुटि मुक्त मूल्यांकन में मदद करेगा।

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NOMENCLATURE AND ABBREVIATIONS

English Notations

a	Contact-radius (m)
E	Elastic Modulus (N/m ²)
F_a	Adhesion force (N)
F_e	Elastic force (N)
F_D	Drag Force (N)
G	Shear modulus (N/m ²)
G^*	Complex shear modulus (N/m ²)
G'	Storage modulus of sample (N/m ²)
G''	Loss modulus of sample (N/m ²)
H_d^*	Hydrodynamic drag force in complex number form (N)
H_d'	Storage modulus of drag (N)
H_d''	Loss modulus of drag (N)
$b(h)$	Drag factor at a height
$b(0)$	Drag factor at contact with sample or surface
f	Linear frequency (Hz)
z	Transverse piezo transducer motion (m)
Z	Transverse distance
A_c	Contact area (m ²)
s	Distance between location of point force and observation point
S	Slant height (m)
h	Sample height at the point of indentation (m)

h	Gap between cantilever tip and substrate or sample surface
$G(s)$	Green's function
$P(r)$	Pressure distribution acting a radial distance 'r'
r	Radial distance
T	Surface traction force
u	Surface displacement
c	Cone
f	Flat cylinder
d	Cantilever deflection
k	Cantilever stiffness
w_a	Work of adhesion
v	Relative velocity
M_{eff}	Effective mass of cantilever
BM-MSCs	Bone marrow-derived stem cells

Greek Notations

δ	Indentation depth (m)
δ_0	Pre-indentation depth (m)
ω	Angular frequency (S^{-1})
γ_a	Adhesion parameter (J/m^2)
θ	Half cone angle
ν	Poisson's ratio
δ/h	Transverse strain

μ Viscosity (N-s/m²)

Ω Surface layer

Abbreviations

AFM Atomic force microscopy

SPM Scanning probe microscopy

SMFS Single molecule force spectroscopy

SCFS Single cell force spectroscopy

FFT Fast Fourier transform

CP Contact point

DP Detachment point

PAF Peak adhesion force (N)

RT Room temperature (°C)

InvOLS Inverted optical lever sensitivity (m/V)

S&D Signal and deflection

MSC Mesenchymal stem cell

HCT-116 It is type of colorectal cancer cell line

LT Tangent modulus