

ICMOBT3 2009 Clearwater, FL

# Evaluating the Mechanical Behavior of a Cell Based on AFM indentation

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# Outline

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- Introduction and Significance
- Objective of our study
- Studying methods
- Basic results and discussions
- Conclusions
- Acknowledgement

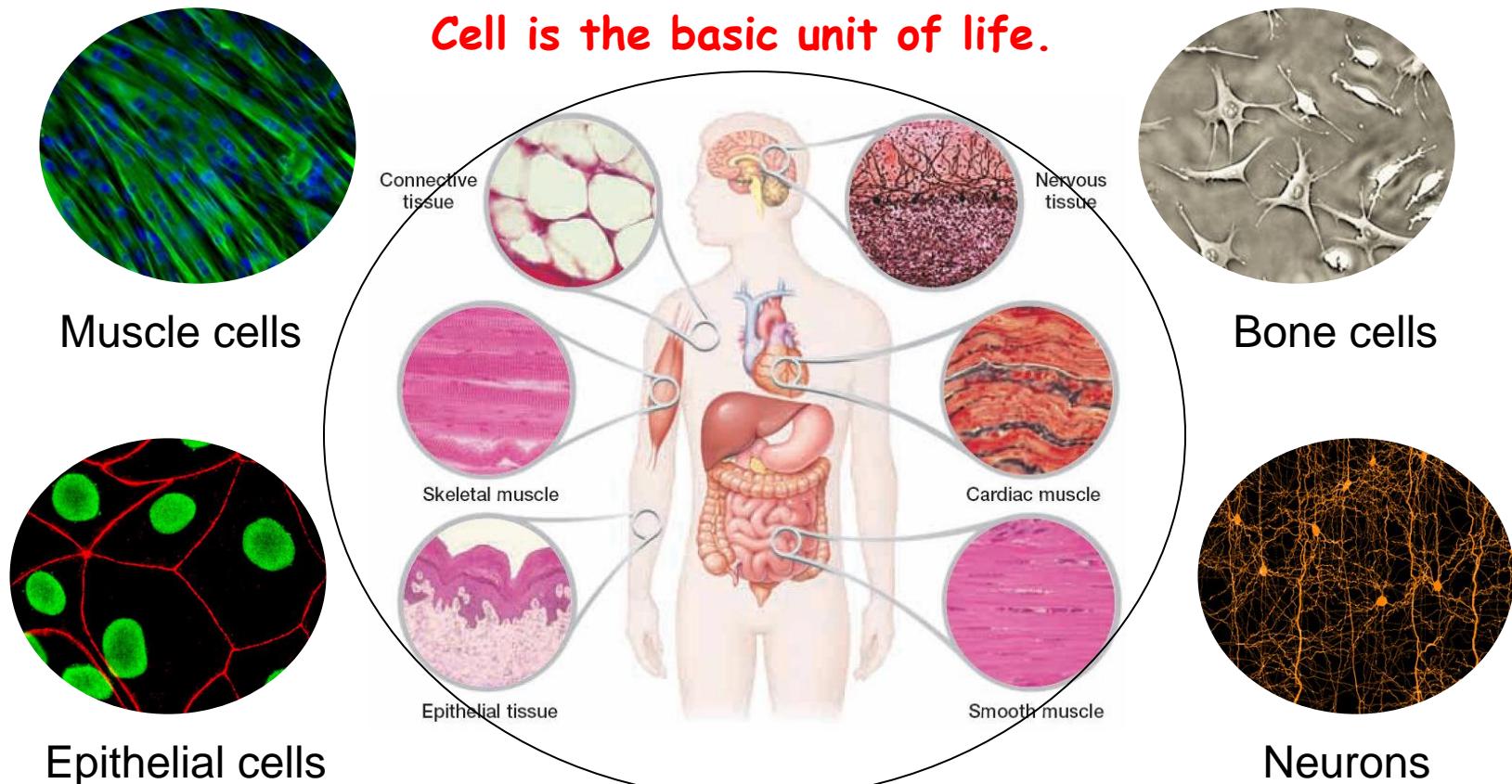


# Introduction and Significance

Human: multiscale biomaterial system



Cell is the basic unit of life.



# Introduction and Significance

Mechanical response  $\leftrightarrow$  Biofunctions

Matters transport between inside and outside, growth, locomotion, disease and death

Cancer	Cell E $\downarrow$ 70%	Cross SE, 2007
Heart failure	Heart muscle Cell contractility $\downarrow$	Feng YC 1993
TBI	Sever stretching $\rightarrow$ neuron death	Morrison B. 1998 Leung LY 2008
Neuron growth	Slow stretching	Smith DH 2001
Cell locomotion	Substrate stiffness	Pelham RJ 1997

- Understand mechanical response  $\leftrightarrow$  bio/chemical response;
- Understand injury/failure mechanism;
- Diagnose the healthy state;
- Build criterion for protection design.

References: Trepat X. et al. *Nature* (2007), Lu YB et al. *PNAS* (2006), Pelling AE et al. *Science* (2004) Zhang PC et al. *Nature* (2001), Ellis BB et al. 1995, Wang JH et al. 2000, Pfister BJ et al. 2003

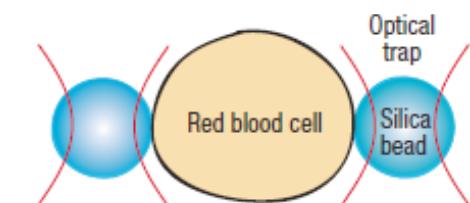
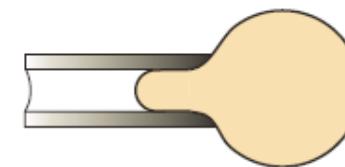
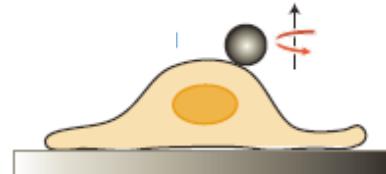
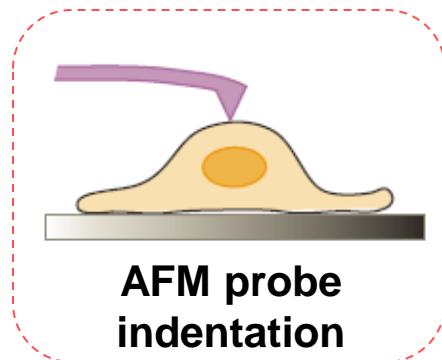


# Introduction and Significance

## Experimental measurement of cell property:

Cell : simplified as continuum, homogeneous, elastic/viscoelastic material

Cell property: fitted from overall force-displacement relationship



- Local deformation
- Anchorage dependent cell:  
cell mounted on substrate

- Global deformation
- Anchorage independent cell

AFM probe indentation: simplest, easiest, most popular method.

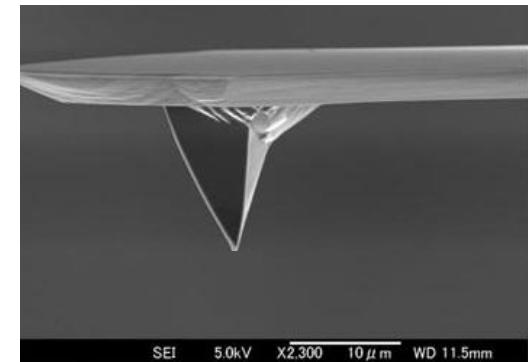


# Introduction and Significance

## Cell Elastic modulus measured by AFM

Cell type	E (kPa)	References
Endothelial cells		
HUVEC	10–11	Sato et al. (2004)
–	1.3–7.2	Mathur et al. (2000)
–	0.9–1.7; 12.0–18	Ohashi et al. (2002)
BPAEC	0.2–2.0	Pesen and Hoh (2005)
Leukocytes		
Leukemia myeloid cells (HL60)	0.2–1.4	Rosenbluth et al. (2006)
Leukemia lymphoid (Jurkat) cells	0.02–0.08	
Neutrophils	0.2–0.07	
Corti organ's cells		
Outer hair cells	300–400	Tolomeo et al. (1996)
Guinea pig's outer hair cells	2–4	Sugawara et al. (2002)
Mouse outer hair cells	2–4	Murakoshi et al. (2006)
Guinea pig's inner hair cells	0.1–0.5	Sugawara et al. (2002)
Hensen's cells	0.3–1.1	–
Osteoblasts	0.3–20.0	Simon et al. (2003)
Astrocytes	2–20	Yamane et al. (2000)
Fibroblasts	4–5	Bushell et al. (1999)
Migrating 3T3 cells	3–12	Rotsch et al. (1999)
–	0.6–1.6	Mahaffy et al. (2004)
L 929	4–5	Wu et al. (1998)
Epidermal keratocytes	10–55	Laurent et al. (2005)
Platelets	1–50	Radmacher et al. (1996)
Skeletal muscle cells		
Murine C <sub>2</sub> C <sub>12</sub> myoblasts	11–45	Collinsworth et al. (2002)
Murine C <sub>2</sub> C <sub>12</sub> myotubes	8–14	Zhang et al. (2004)
–	10–17	–
–	28–21	Mathur et al. (2001)
Myofibrils	40–45	Yoshikawa et al. (1999)
Cardiocytes	90–110	Mathur et al. (2001)
Rat	32–42	Lieber et al. (2004)
Chicken	5–200	Hofmann et al. (1997)
Erythrocytes		
	14–18	Mozhanova et al. (2003)
	19–33	Dulinska et al. (2006)
	22–64	–
	16–64	–
	70–110	–

Standard AFM  
Indentation: conical tip

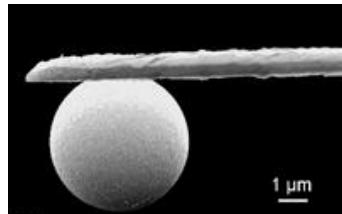


- Sharp to damage the cell membrane;
- Introduce high stress/strain under tip—nonlinear behavior.
- Contact radius very small—high fluctuation

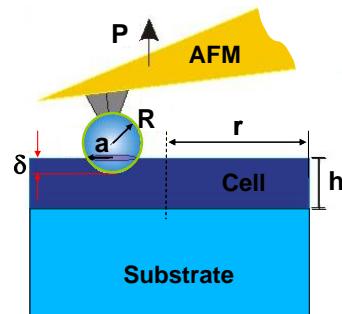


# Introduction and Significance

AFM Indentation with Spherical tip: larger contact area, lower stress



Spherical tip



Spherical indentation

Glue a sphere (glass or polymer) with  $d = 1 \sim 10 \mu\text{m}$

The cell modulus is typically determined by:

**Hertz contact model** with spherical tip:

$$\text{Quasistatic indentation : } P = \frac{4}{3} \frac{E \sqrt{R} \delta^{\frac{3}{2}}}{1 - \nu^2}$$

Dynamic indentation:

$$\delta(t) = \delta_0 + \Delta\delta \sin(\omega t), \quad P(t) = P_0 + \Delta P \sin(\omega t + \phi)$$

$$\frac{E'(\omega)}{1 - \nu^2} = \frac{\Delta P}{\Delta \delta} \frac{1}{2\sqrt{R\delta}} \cos \phi \quad \frac{E''(\omega)}{1 - \nu^2} = \frac{\Delta P}{\Delta \delta} \frac{1}{2\sqrt{R\delta}} \sin \phi$$

References based on spherical indentation:

Callies C., 2009, (endothelial cells); Carl P., 2008, (cho cells); Radmacher M. 2007, (eukaryotic cells); Hansen JC, 2006, (osteoblastic cells); Lu YB, 2006, (glial, neurons); Lulevich V, 2006, (lymphocyte); Rico F, 2005 (epithelial cells); Mahaffy RE, 2004, (fibroblasts).

# Objective of our study

Hertz solution is for "elastic contact of semi-infinite space".

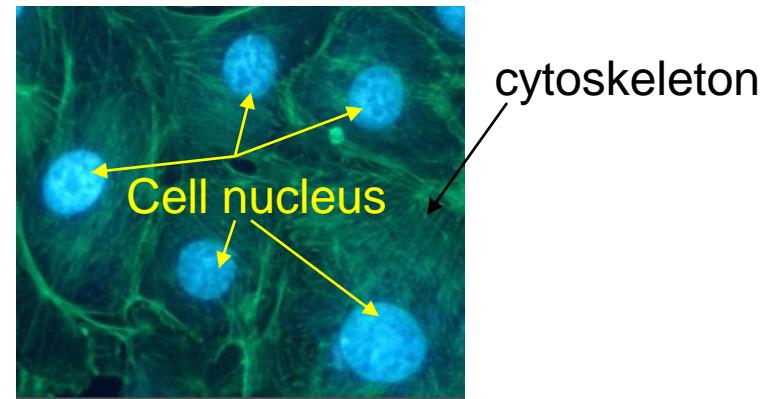
conditions:

- (1) Isotropic, homogeneous material;
- (2) Very small surface curvature;
- (3) Indentation displacement  $\delta \ll h$ ;
- (4)  $\delta \ll R$ .

**Problems:** all those conditions are not met for AFM indentation on cells

Cytoskeleton: reported elastic modulus is around GPa level, provide the main mechanical properties of cells.

Nucleus: 3~12 times stiffer than cytoplasm from reference.



Cells: shell-core structure

**Objectives:**

- (1) subcellular effect on spherical indentation behavior of cell;
- (2) validation of conventional indentation analysis.

# Studying Method (FEM)

## I. Computational model of nucleus effect:

### **Reference reported results:**

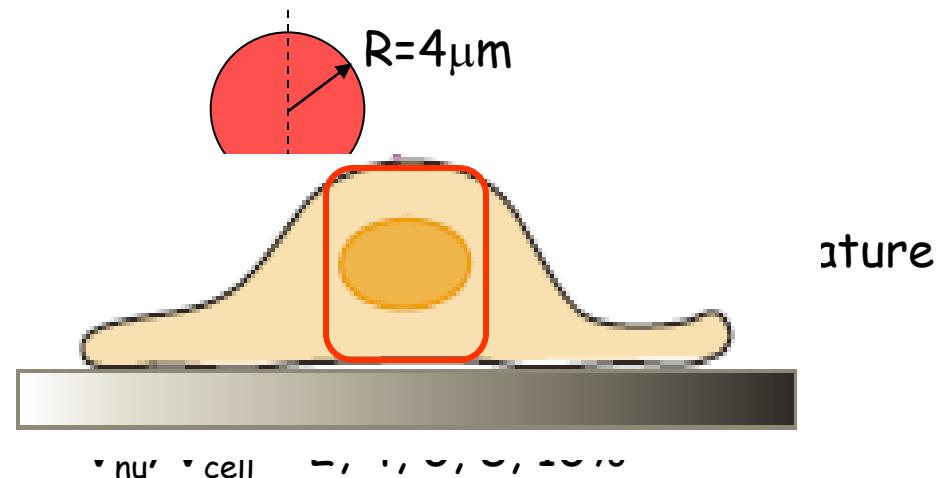
Based on endothelial,  
chondrocyte, neutrophil,  
epithelium and smooth muscle  
cell:

**Material:** linear elastic/linear viscoelastic model

$$E_{nu} = 3 \sim 15 E_{cyto}, \mu_{nu} \approx 2 \mu_{cyto}$$

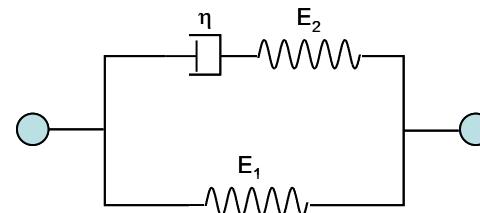
**Geometry:** axial symmetric structure  $h = 5.6\text{-}15\mu\text{m}$ ,  $d = 9.4\text{-}21.1\mu\text{m}$  and  $V_{\text{nu}}/V_{\text{cell}} \leq 10\%$

## Geometric model: (axial symmetric)



## Material model: linear viscoelastic

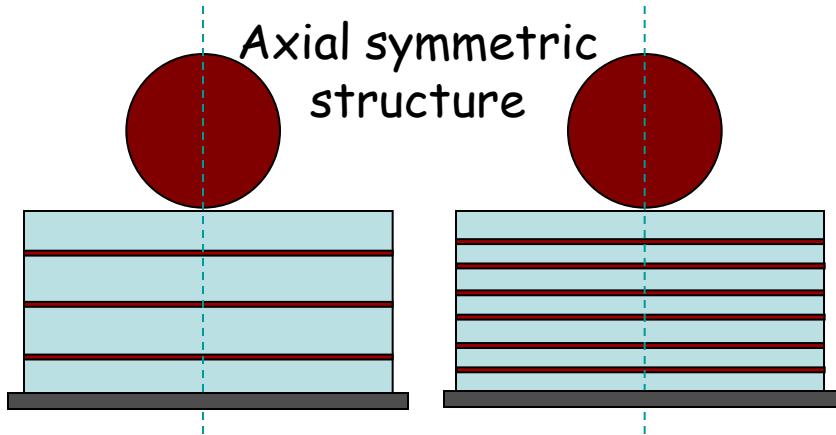
$$E_{nu} = 2 \sim 16 E_{cyto} \quad \mu_{nu} \approx 2 \mu_{cyto}$$



# Standard linear solid

# Studying Method (FEM)

## II. Computational model of cytoskeleton effect:



**Material:** linear elastic material

$$E_s \gg E_p \rightarrow E_s = 10 \sim 1000 E_p$$

**Geometry:** has similar dimension as the last one with nucleus

$$V_s = 6 \sim 12\% V_{cell} \quad \text{Number: } n = 3 \sim 6$$

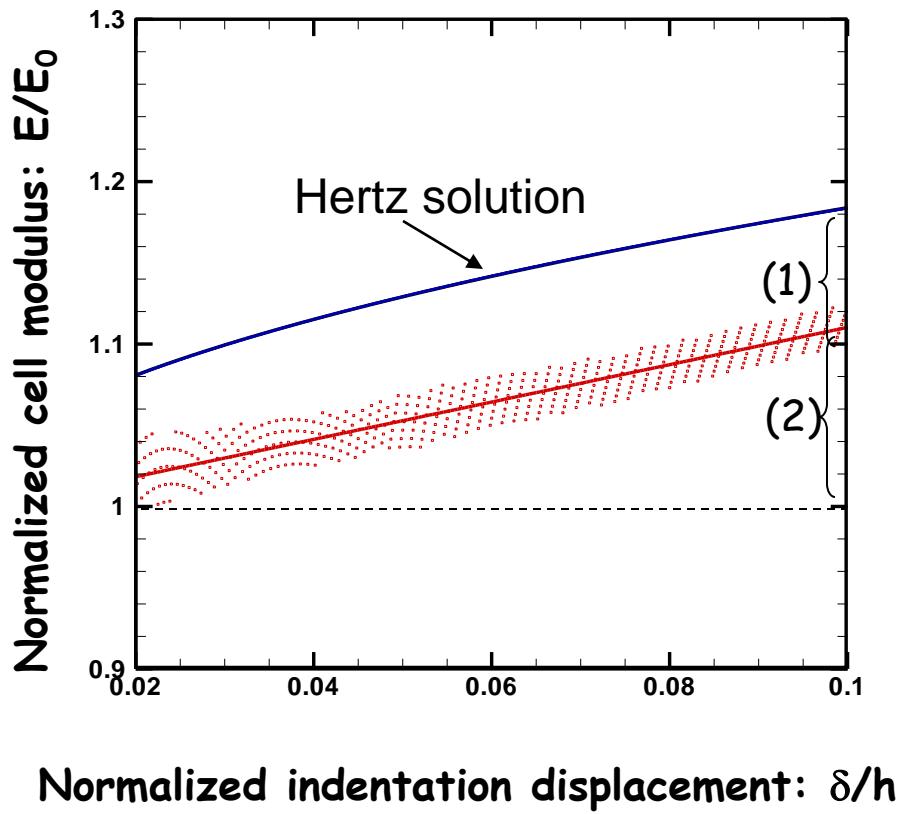
## Results:

Simulations of Nucleus and cytoskeleton effects on:

- (1) cell modulus based on the Hertz's solution (conventional indentation analysis);
- (2) indentation contact radius;
- (3) Substrate stiffening effect;
- (4) Cell modulus after decoupling the effects of contact radius and substrate stiffening.

# Cell Property from Homogeneous Model

$E_0$ : real cell modulus



For cell with homogeneous model:

$$\text{Conventional solution: } E = \frac{3}{4} \frac{P_{\text{num}}(1-\nu^2)}{\sqrt{R}\delta^{\frac{3}{2}}}$$

Problems:

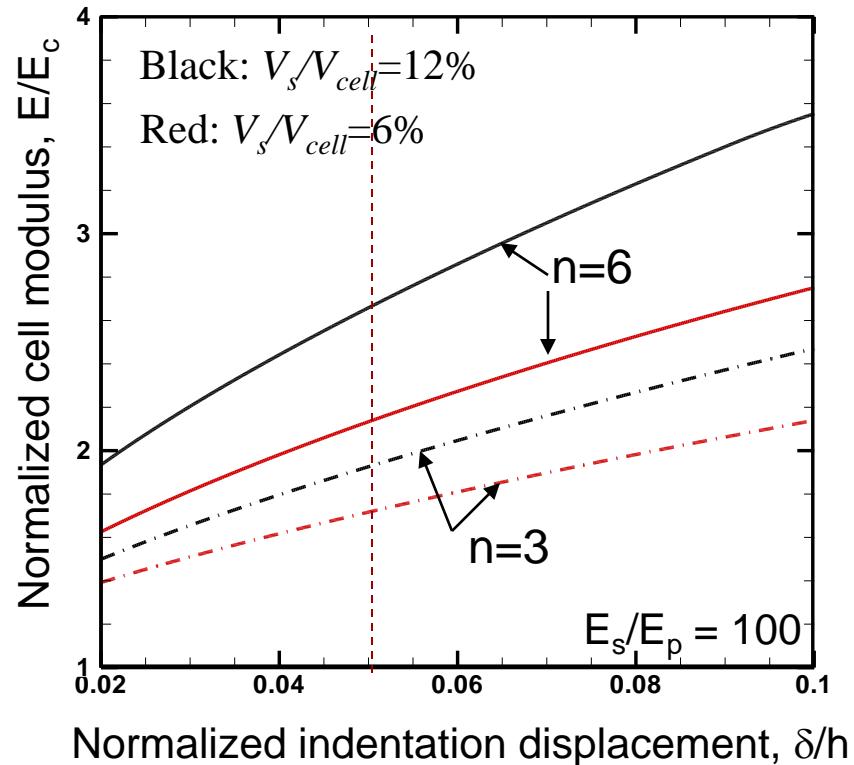
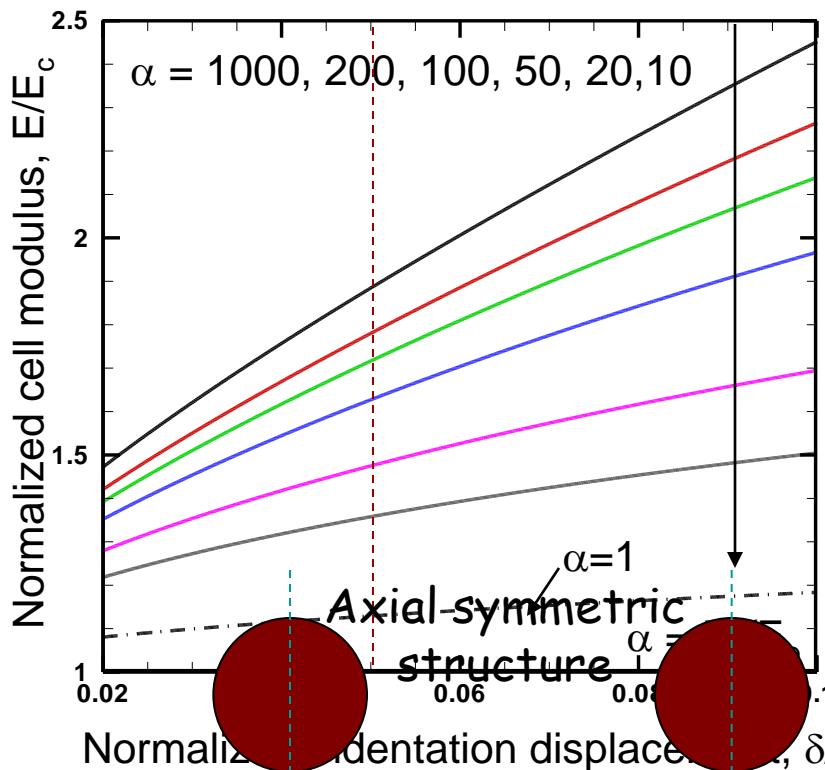
- (1) Hertz contact radius is less than the computed one :  $a_{\text{FEM}} > a_0$ ;
- (2) Substrate interferes the indentation stress field to stiffen the cell.

Results:

Cell modulus is overestimated for 18%; about 10% from  $a_{\text{FEM}} > a_0$ ; the rest from substrate stiffening effect.

# Cytoskeleton effects

Cytoskeleton effect on cell modulus based on the Hertz solution

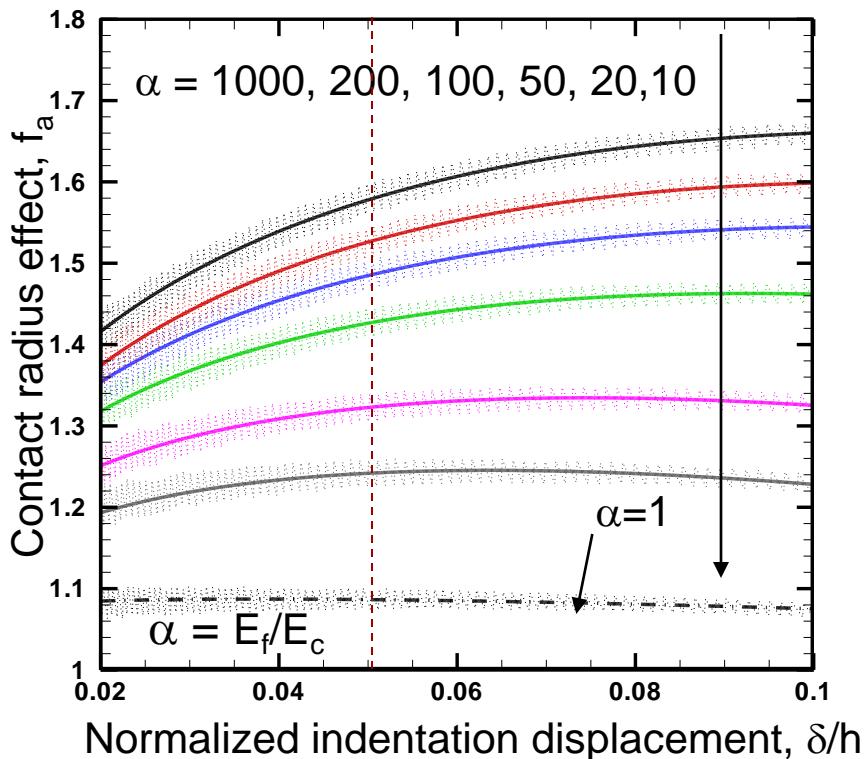


Varying # and volume fraction of cytoskeleton,

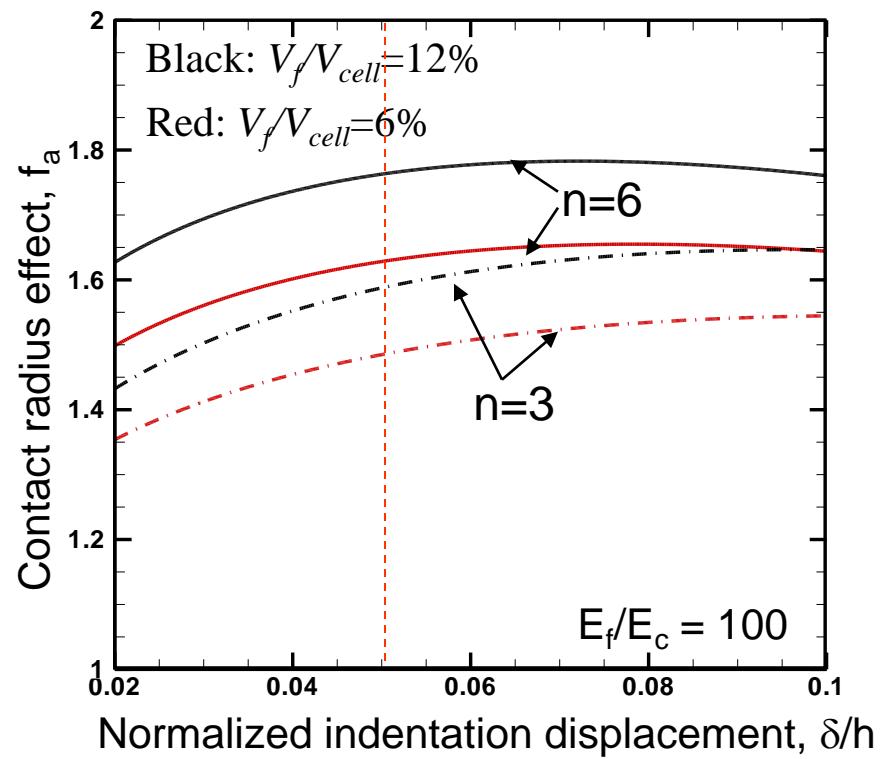


# Cytoskeleton effects

$$E \propto a^3 \quad \rightarrow \quad \text{Contact radius effect: } f_a = (a_{\text{num}}/a_{\text{Hertz}})^3$$



Varying cytoskeleton modulus,  
with  $n=3$  and  $V_s/V_{\text{cell}} = 6\%$

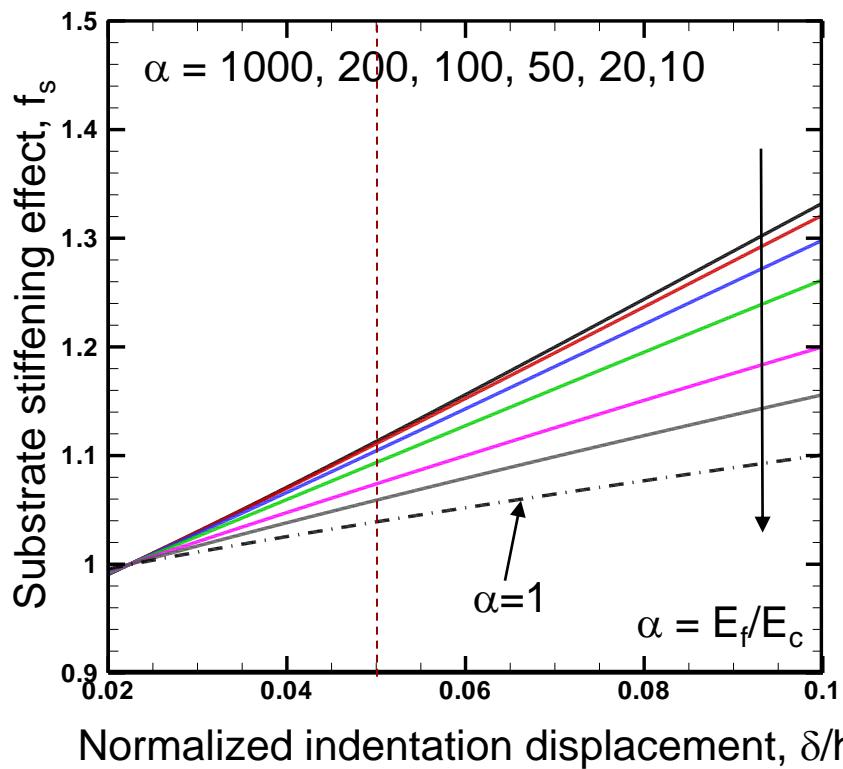


Varying # and volume fraction  
of cytoskeleton,

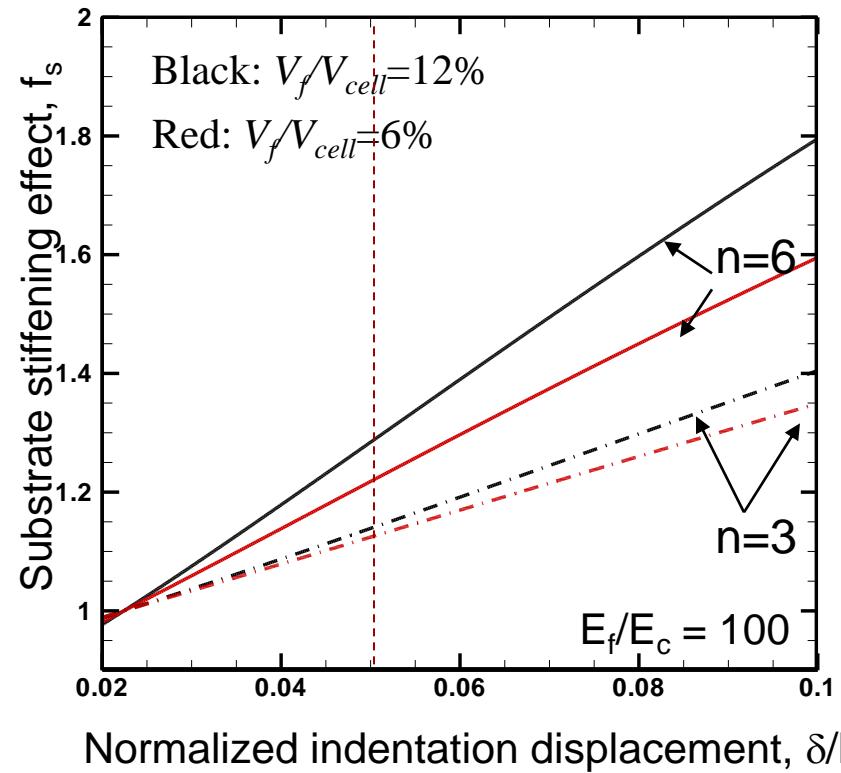
# Cytoskeleton effects

**Substrate stiffening effect** scales with the stress at the bottom of cell  $\varepsilon_b$

Substrate stiffening effect:  $f_s - 1/f_{s0} - 1 = \varepsilon_b / \varepsilon_{b0}$ ;  $f_{s0}, \varepsilon_{b0}$ : homogeneous model;



Varying cytoskeleton modulus,  
with  $n=3$  and  $V_s/V_{cell} = 6\%$



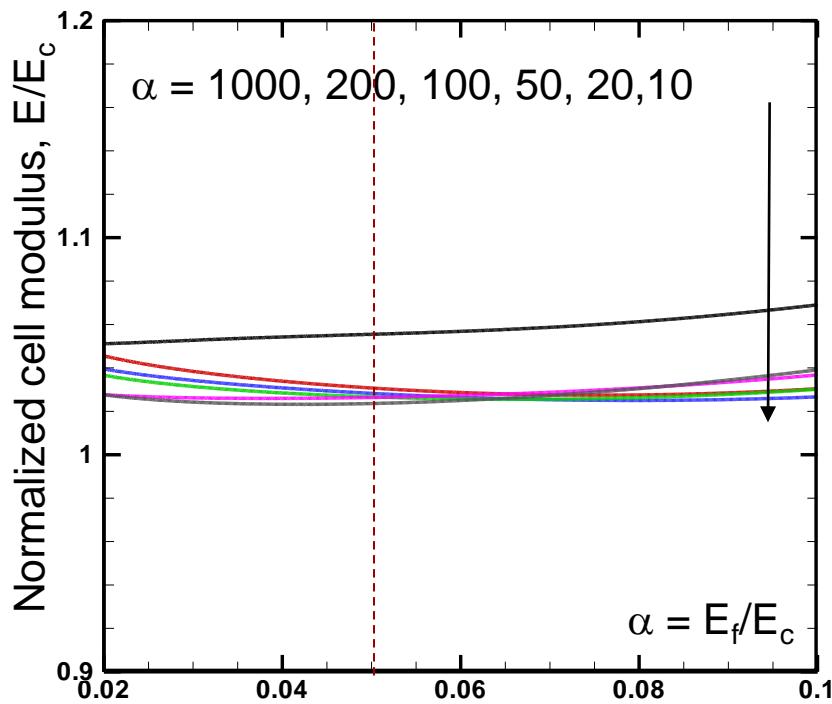
Varying # and volume fraction  
of cytoskeleton,

# Cytoskeleton effects

Decoupling the effects of substrate and contact radius:

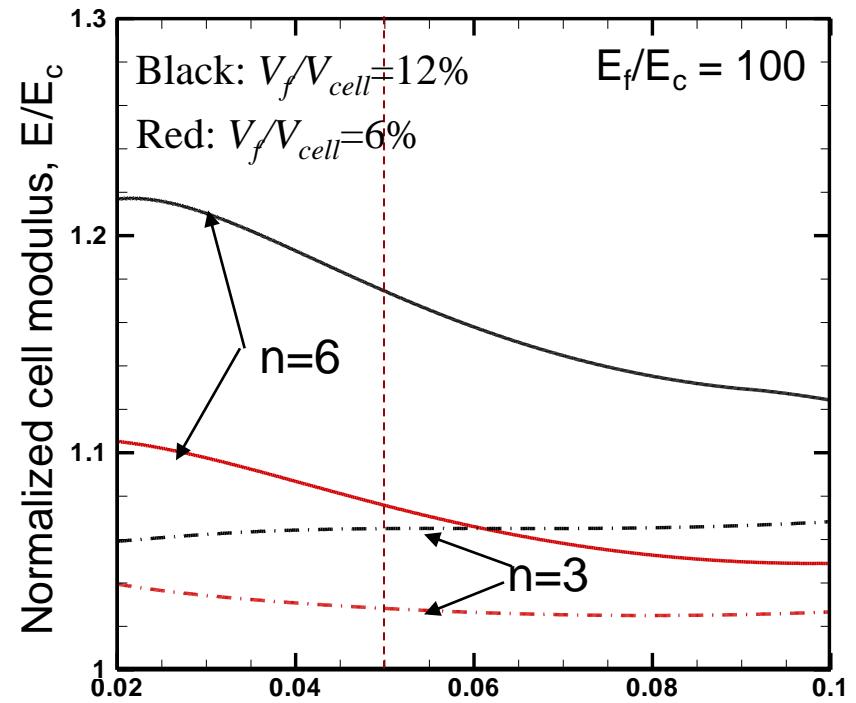
$$E = E_0 f_s f_a f_s$$

$f_s$ : cytoskeleton effect  
 $E_0$ : cytoplasm modulus



Normalized indentation displacement,  $\delta/h$

Varying cytoskeleton modulus,  
with  $n=3$  and  $V_s/V_{cell} = 6\%$



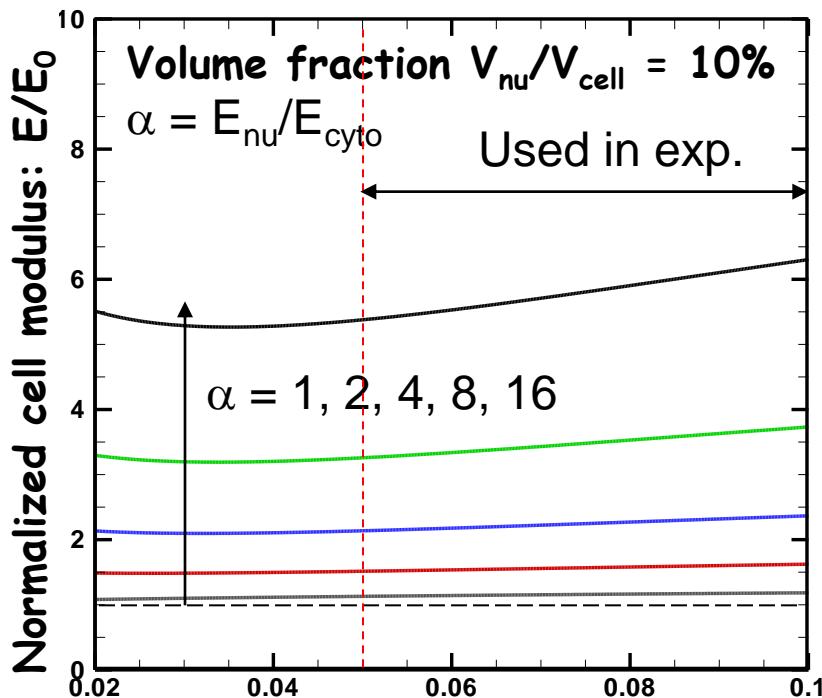
Normalized indentation displacement,  $\delta/h$

Varying # and volume fraction  
of cytoskeleton,

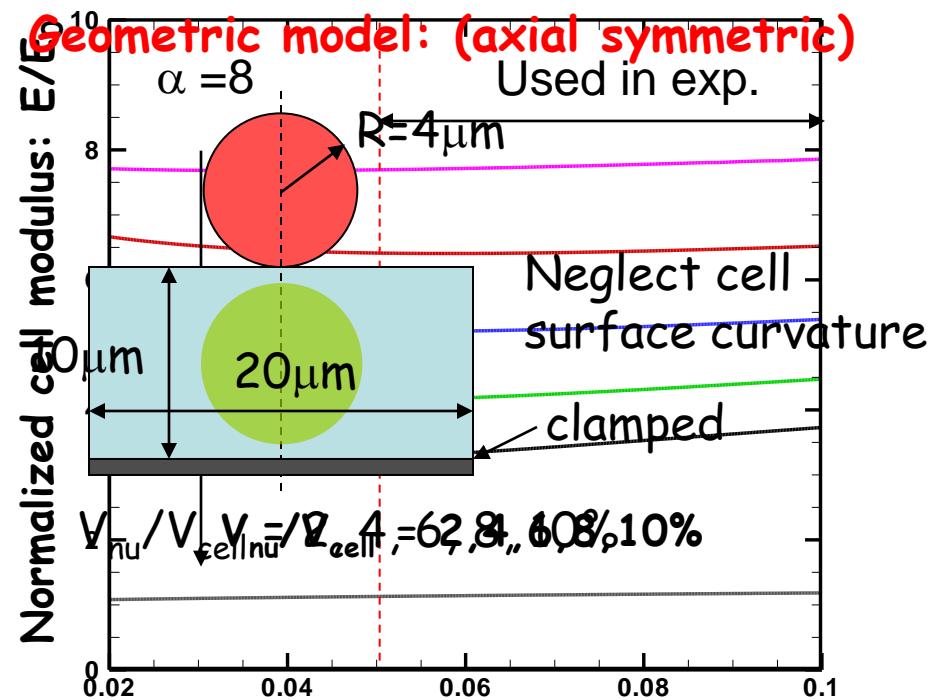


# Nucleus effect

Nucleus effect based on Hertz's solution



The effect of nucleus modulus

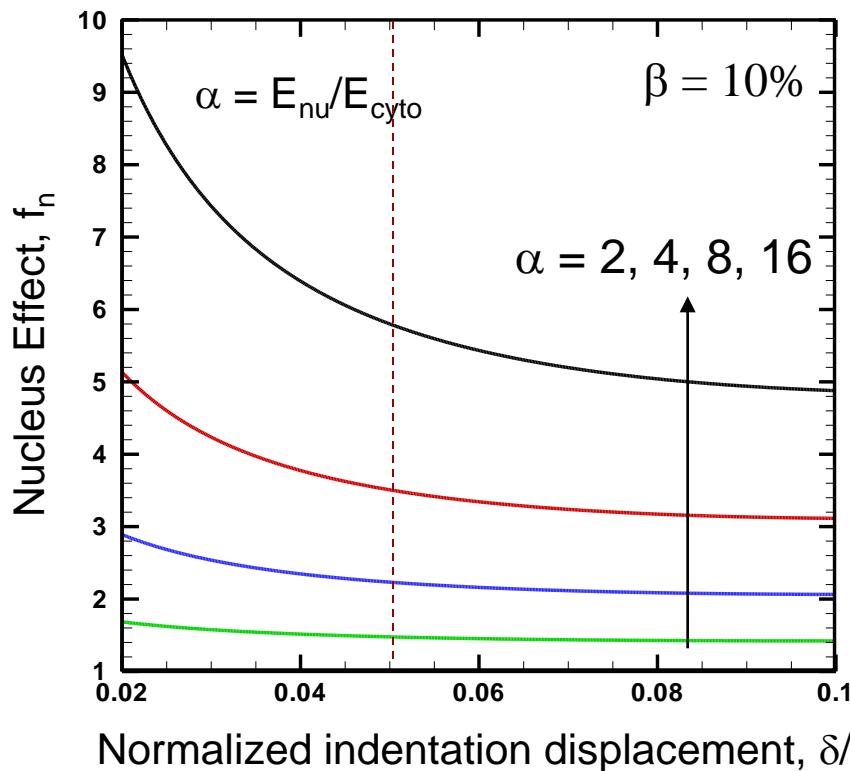


The effect of nucleus volume fraction

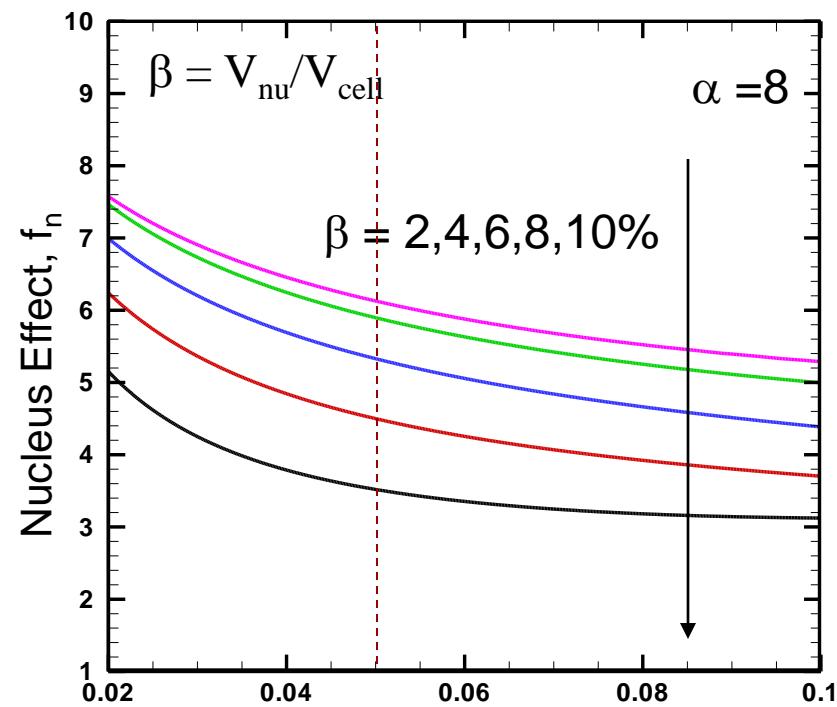
# Nucleus effect

Heterogeneous model:  $E = E_0 f_s f_a f_n$

$f_n$ : nucleus effect  
 $E_0$ : cytoplasm modulus



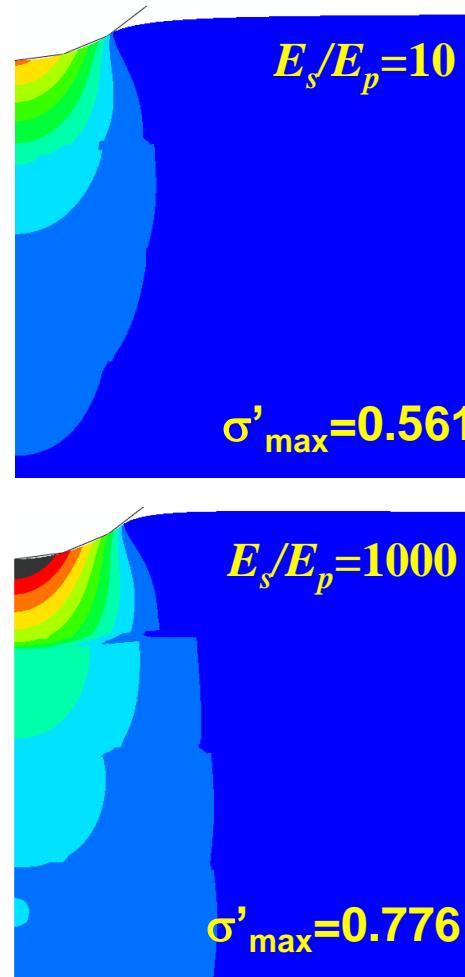
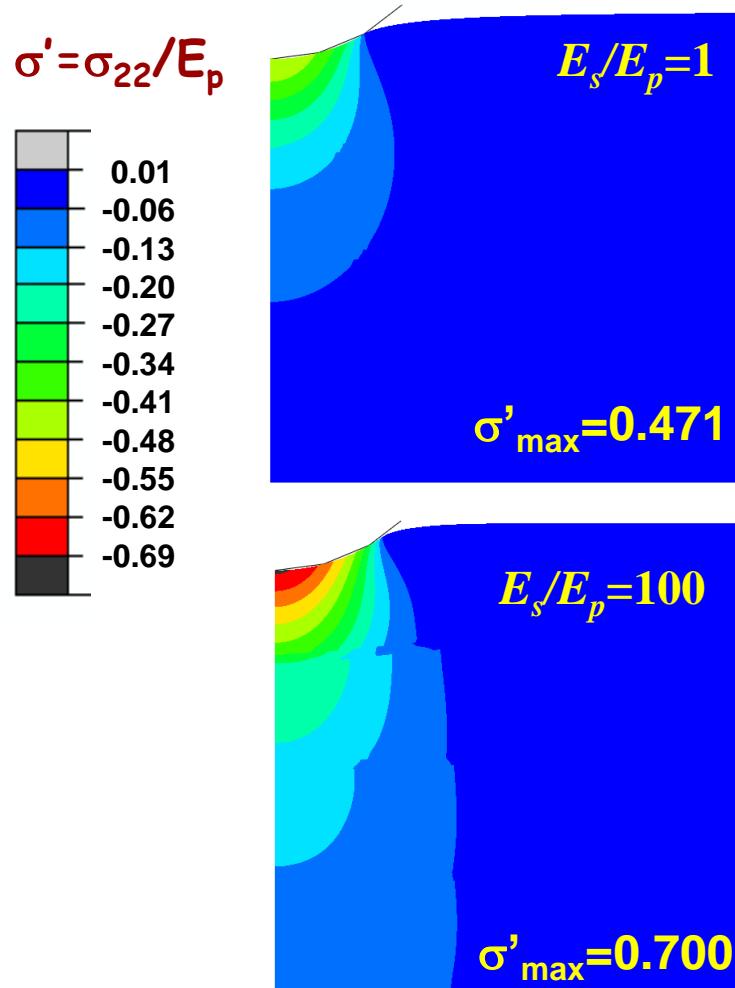
The effect of nucleus modulus



The effect of nucleus volume fraction

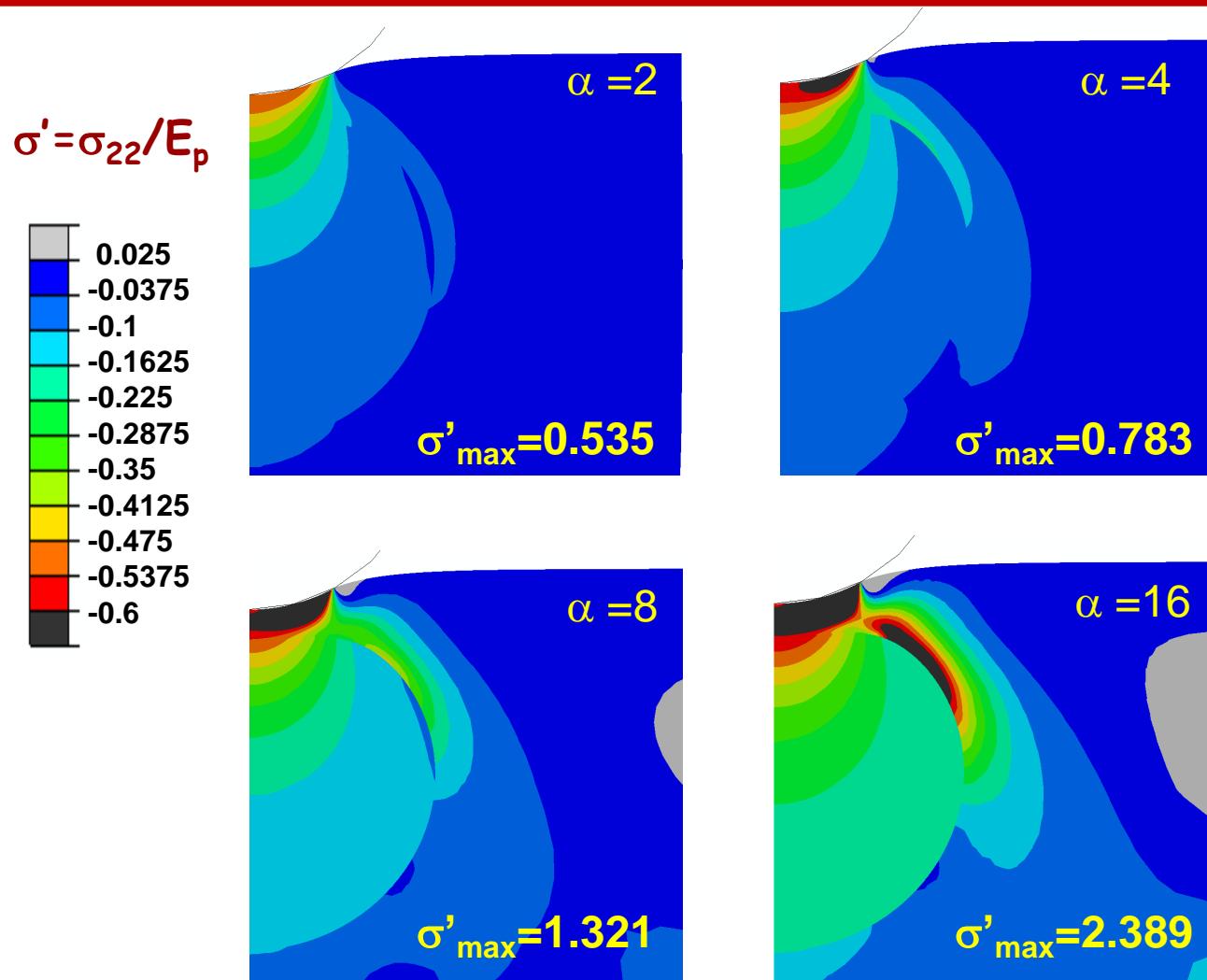


# Cytoskeleton effect on indentation stress $\sigma_{22}$



Cytoskeleton with the same  
 $V_s/V_p = 6\%$   
 $n_s = 3$   
Modulus increases  
 $E_s/E_p = 1 \sim 1000$

# Nucleus effect on indentation stress $\sigma_{22}$



Nucleus with  
the same

$$r_{nu} = 4.22$$

$$V_{nu}/V_{cell} = 10\%$$

Modulus increases

$$E_n/E_p = 2 \sim 16$$

# Indentation stress $\sigma_{22}$ Animation

## Animation of $\sigma_{22}$

- (1) Pure cytoplasm
- (2) With cytoskeleton  
 $V_s/V_{cell}=6\%$ ,  $E_s/E_p=100$
- (3) With nucleus  
 $V_n/V_{cell}=8\%$ ,  $\alpha=8$

Without nucleus



$\alpha = 8$

$V_{nu}/V_{cell}=8\%$

# Conclusions

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1. Cell mechanical properties are strongly dependent on the measuring method. Under spherical indentation, cell modulus can be significantly increased with a stiffer nucleus; while cell modulus is not sensitive with the cytoskeleton.
2. The Hertz contact radius can introduce much larger error than it in the homogeneous model. The substrate stiffening effect is also much stronger in the heterogeneous model.
3. Based on the conventional indentation analysis, with  $V_n/V_{cell} = 2\sim 10\%$ ,  $E_n/E_p = 2\sim 16$ , the nucleus effect can cause the cell overall modulus to vary in the range of 1.18~7.8 times. After correcting the effects of contact radius and substrate, it increases by as high as 6 times.
4. Based on the conventional indentation analysis, with  $V_s/V_{cell} = 6\sim 12\%$ ,  $E_n/E_p = 10\sim 1000$ , the cytoskeleton effect can cause the cell overall modulus to vary in the range of 1.3~3.6 times. After correcting the effects of contact radius and substrate, it increases by only 15%.



# Future Works

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1. Consider the real cell shape, like the partial ellipsoidal shape;
2. Introduce the real 3D simulations.
3. Consider the real nucleus shape, like ellipsoidal shape;
4. Introduce the cytoskeleton into the 3D cell model;
5. Comparing cell properties determined under different method: tensile, compression, magnetic twisting and AFM indentation.



# Acknowledgement

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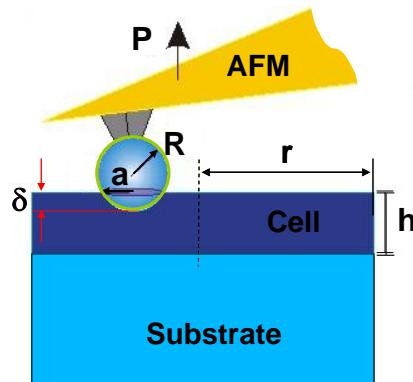
The authors acknowledge the financial support provided by the U.S. Army Research Office (Project Monitor: Bruce Lamattina) for the project "Army- UNL Center for Trauma Mechanics," contract number W911NF-08-1-0483.



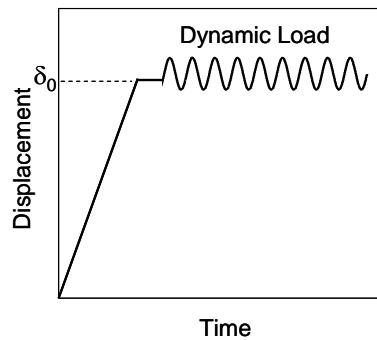
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The End

# Conventional indentation analysis



## Spherical indentation



## Dynamic loading profile

The **Hertz contact model** with spherical tip:  $a \approx \sqrt{R\delta}$

Quasistatic indentation :

$$\text{Elastic model: } P = \frac{4}{3} \frac{E \sqrt{R\delta}^{\frac{3}{2}}}{1 - \nu^2}$$

Viscoelastic model:

$$P(t) = \frac{4}{3} \frac{\sqrt{R}}{1 - \nu^2} \int_0^t E(s) \frac{d\left(\delta(t-s)^{\frac{3}{2}}\right)}{ds} ds$$

Assumptions:  
 (1)  $\delta \ll h$ ;  
 (2)  $\delta \ll R$ .

Local cell deformation

Dynamic indentation:

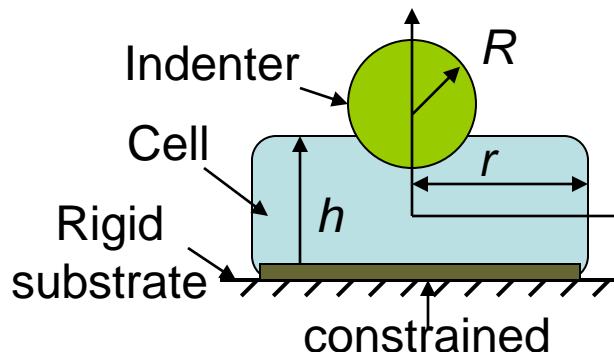
$$\frac{E'(\omega)}{1 - \nu^2} = \frac{\Delta P}{\Delta \delta} \frac{1}{2a} \cos \phi \quad \frac{E''(\omega)}{1 - \nu^2} = \frac{\Delta P}{\Delta \delta} \frac{1}{2a} \sin \phi$$

$$\delta(t) = \delta_0 + \Delta \delta \sin(\omega t), \quad P(t) = P_0 + \Delta P \sin(\omega t + \phi)$$

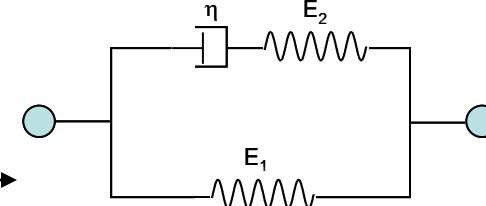
Elastic and Viscoelastic properties of cell can be determined using AFM indentation

# Homogeneous cell model

**Geometric model:**



**Material model:**



Standard linear solid

**Computational model:**



Cell indentation:

- (1) Indenter tip size:  $R=1\sim40\mu\text{m}$ ;
- (2) Cell radius:  $r=10\sim20\mu\text{m}$ ;
- (3) Cell thickness:  $h=5\sim10\mu\text{m}$ ;
- (4) Rigid Substrate;
- (5) Deep indentation;  $\delta=0.5\sim1\mu\text{m}$ ;

**Geometric effect:**

- Indenter tip size
- Cell radius
- Cell thickness (substrate)

**Substrate stiffening effect:**

**Not valid**

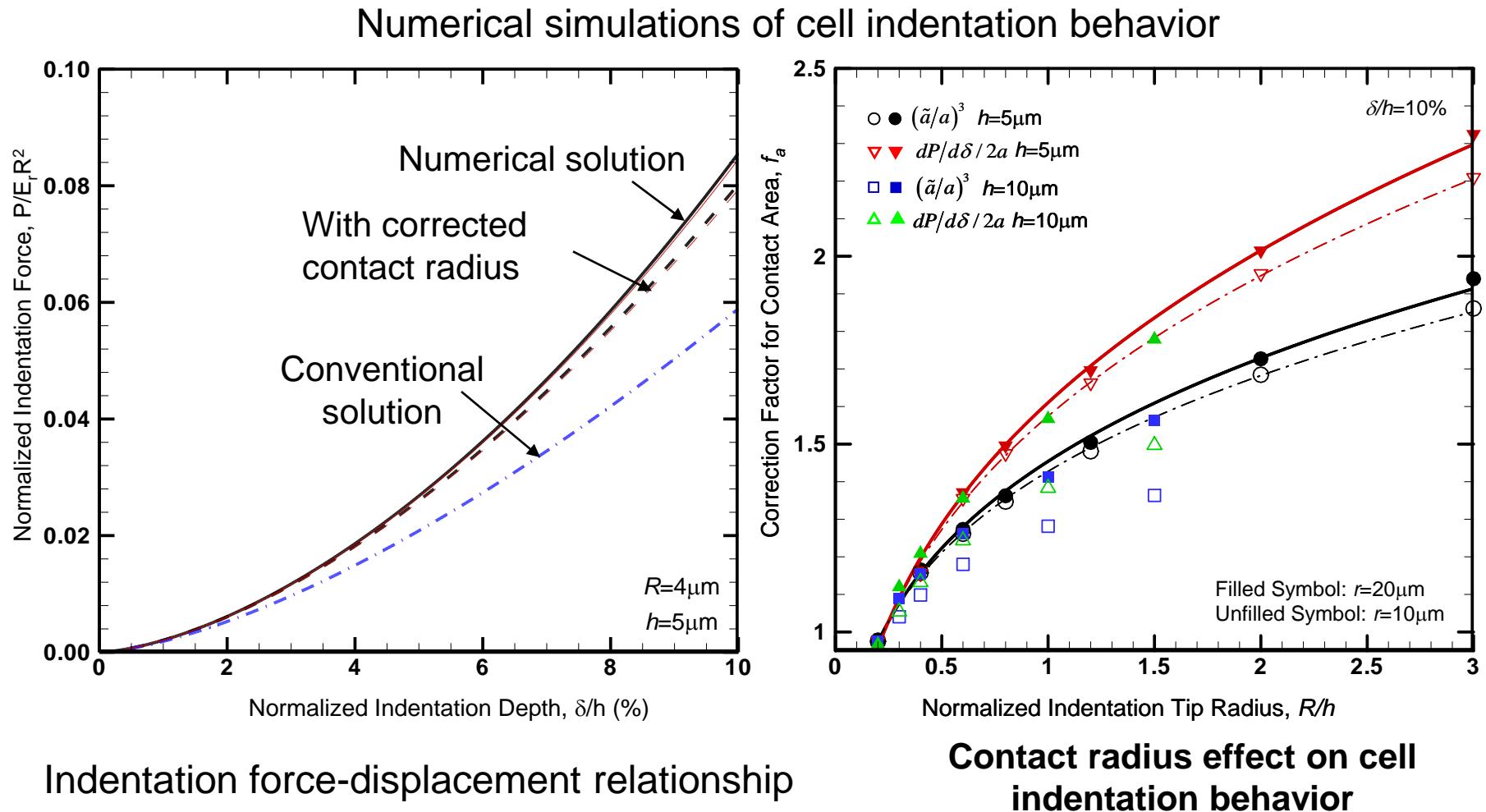
The Hertz model  
 $\delta \ll h, \delta \ll R$

$$a \approx \sqrt{R\delta}$$

$$P = \frac{4}{3} \frac{E \sqrt{R\delta^2}}{1 - \nu^2}$$

Conventional indentation analysis is not valid for biological cell measurement.

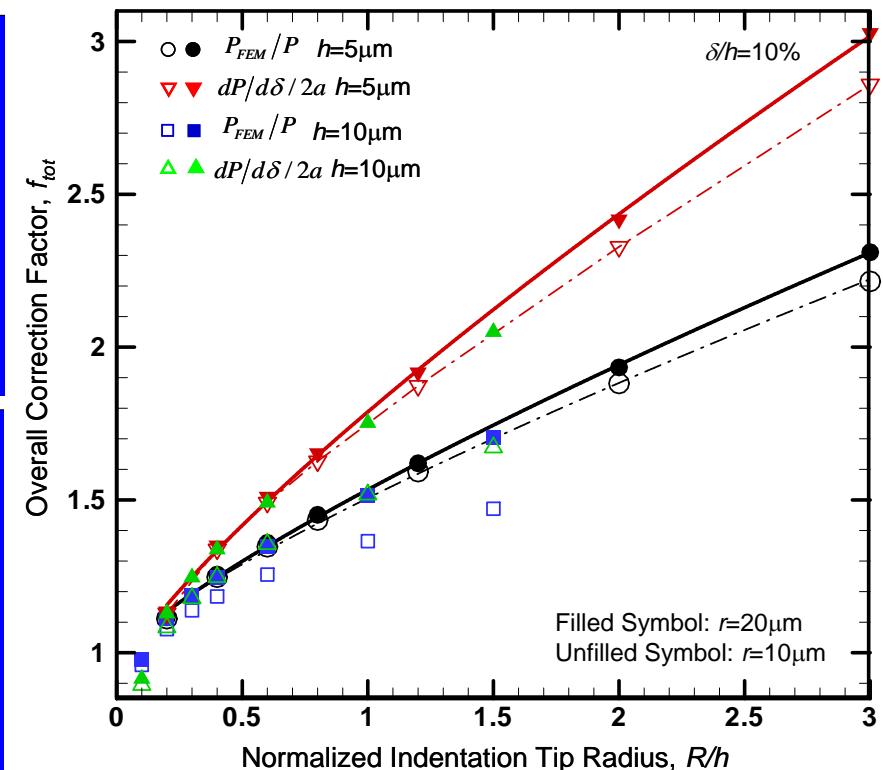
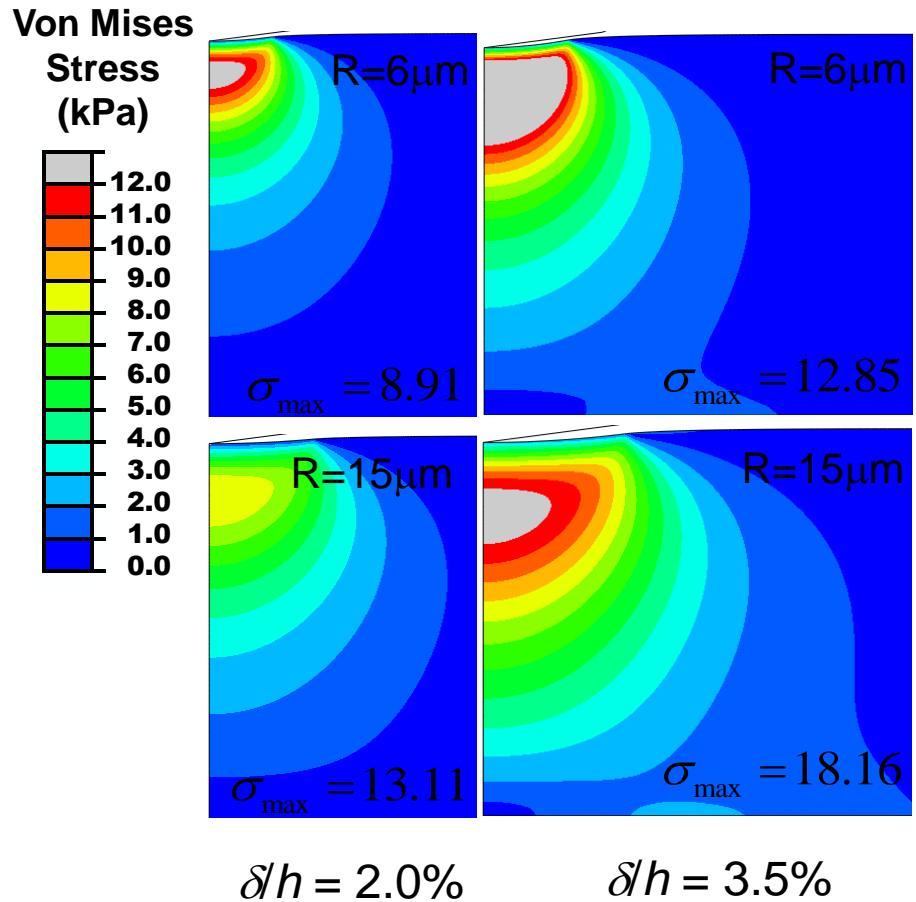
# Contact radius effect on cell indentation behavior



Underestimating contact radius → Overestimating elastic modulus 130%



# Substrate stiffening effect on indentation behavior of cell



Overestimation of cell properties using conventional analysis



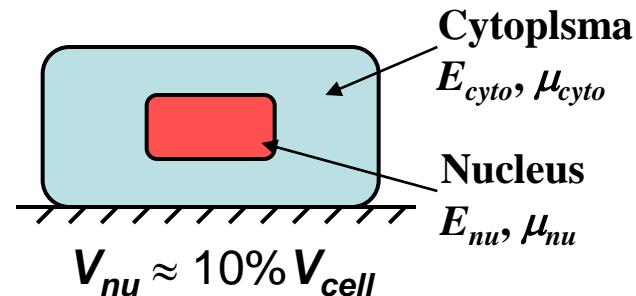
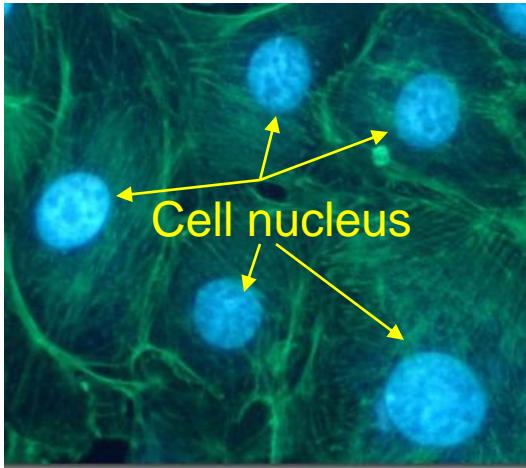
Underestimating contact radius



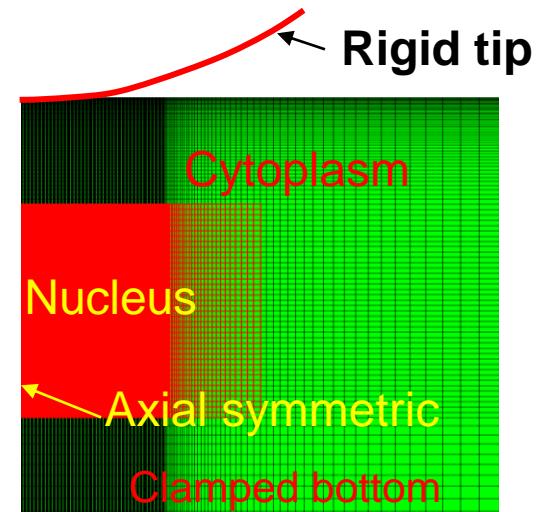
Neglecting Substrate stiffening

Overall overestimation of E of 200%

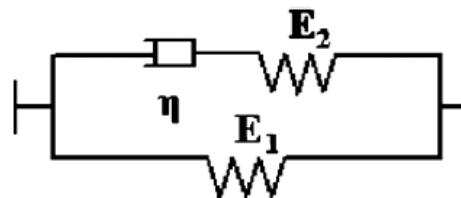
# Heterogeneous cell model



**Heterogeneous cell model**



**Computational model**



**Cytoplasm, nucleus: SLS model**

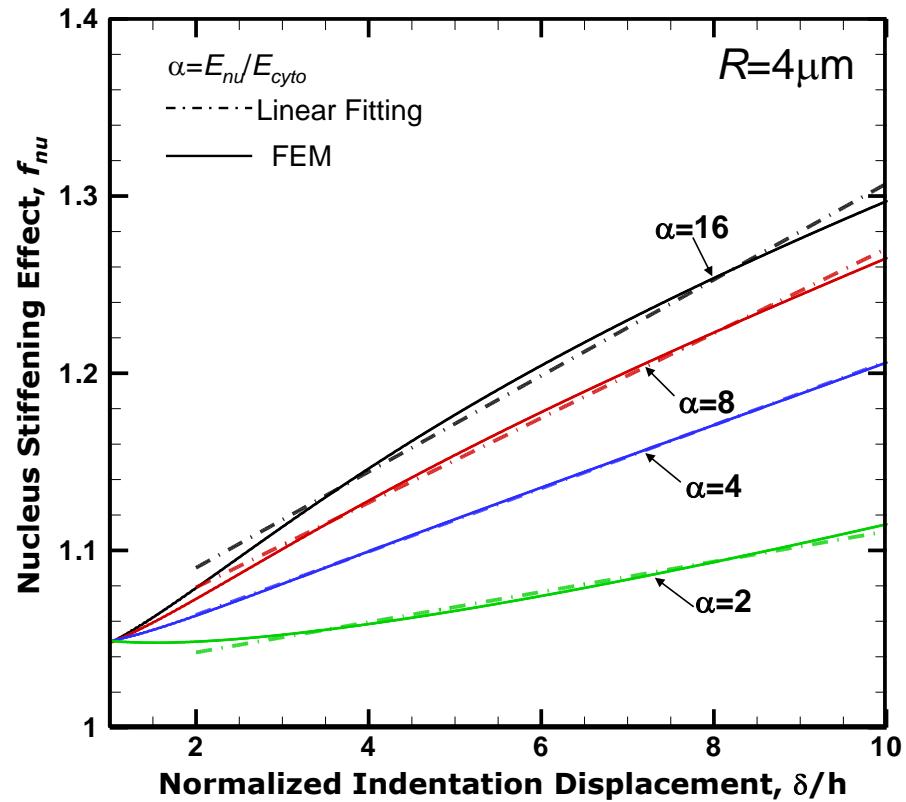
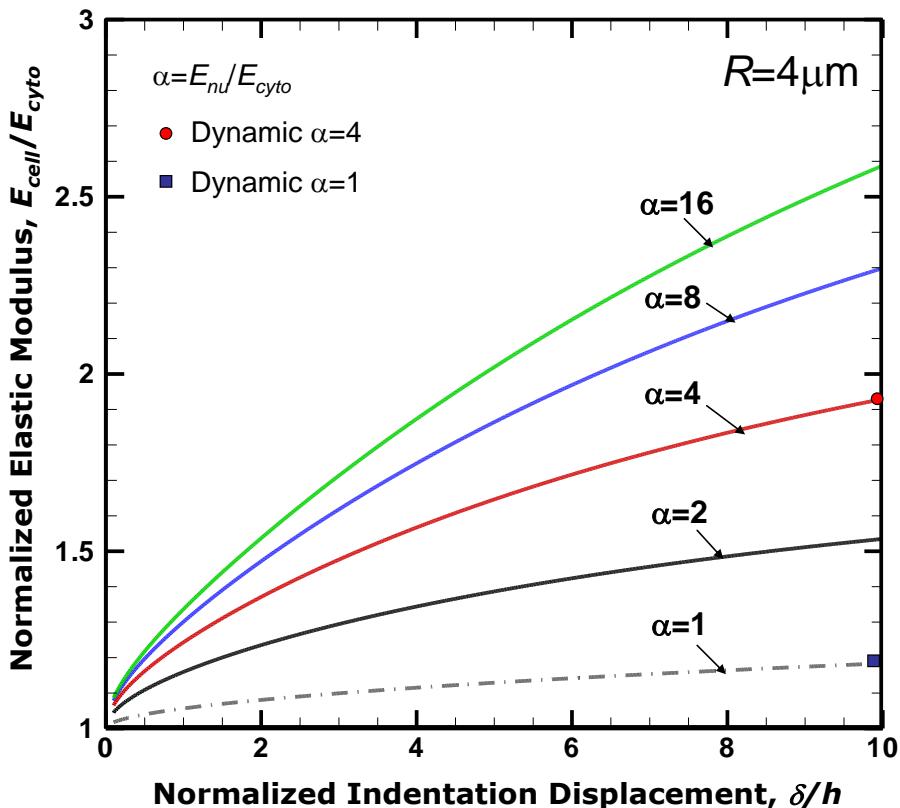
Nucleus: stiffer, more viscous

$$E_{nu} = E_1 + E_2 = 3 \sim 15 E_{cyto} \quad \mu_{nu} \approx 2 \mu_{cyto}$$

**The effects of nucleus on:**

- (1) Indentation displacement – contact radius relationship;
- (2) Substrate effect;

# Nucleus effect on indentation behavior



Conventional Indentation analysis based on Hertz model:

$$E_{cell} = 2.6 E_{cyto} \text{ if } E_{nu} = 16 E_{cyto} \text{ at } \delta/h = 10\%$$

After removing substrate effect and correcting contact radius:

$$E_{cell} = 1.3 E_{cyto} \text{ if } E_{nu} = 16 E_{cyto}$$



Convention analysis can cause much higher overestimation of cell modulus from heterogeneous structure.

# Substrate effect of Heterogeneous Model

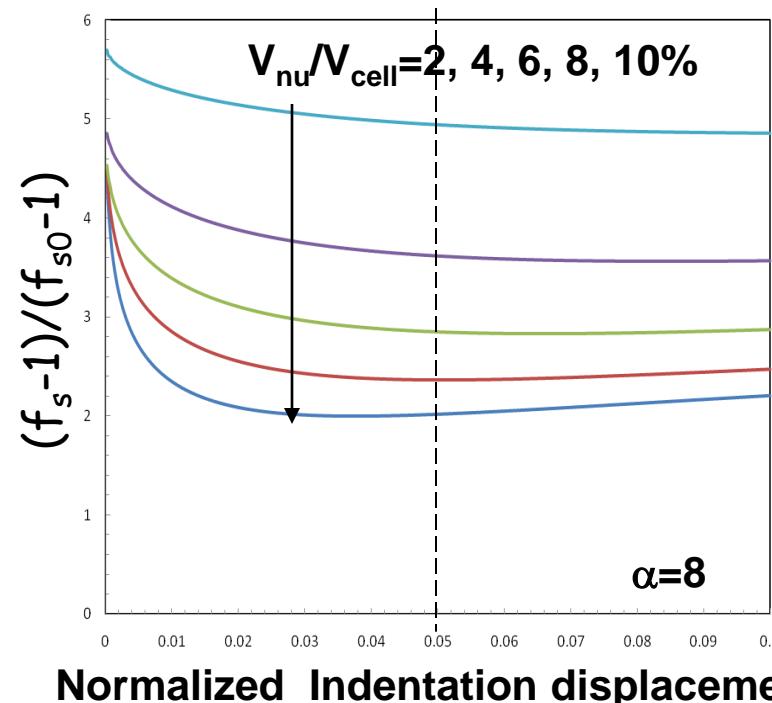
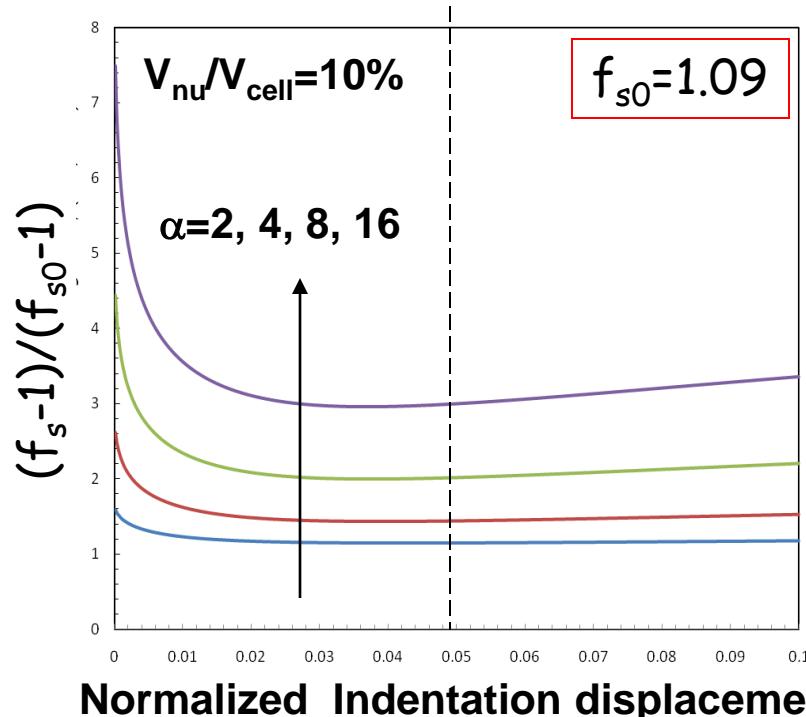
Contribution from  $a_{FEM} > a_0$ :  $f_a = (a_{FEM}/a_0)^3$ .

Homogeneous model:

$$E = E_0 f_{s0} f_{a0}$$

**Substrate stiffening effect scales with the stress at the bottom of cell  $\varepsilon_b$**

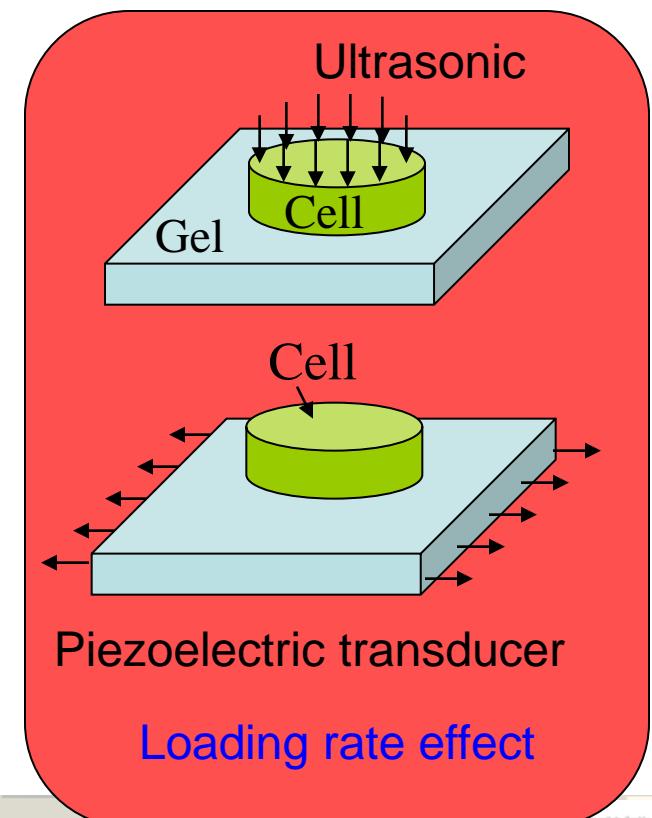
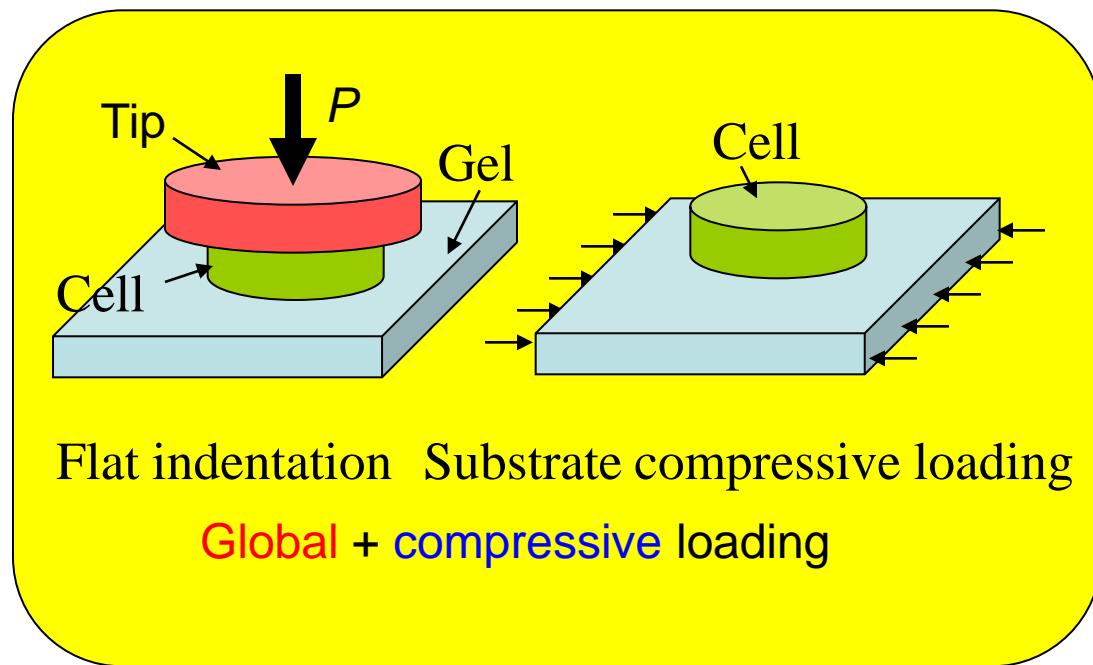
Substrate stiffening effect:  $f_s/f_{s0} = \varepsilon_b/\varepsilon_{b0}$ ;  $f_{s0}, \varepsilon_{b0}$ : homogeneous model;



# Current and planed work

Building cell model to simulate the blast response at cellular level based on both experiments and numerical simulations:

- Cell mechanical behavior under compressive loading
- Response time of cytoskeleton remodeling
- Cell mechanical behavior under global loading



# Indentation stress $\sigma_{22}$ distribution

