FACULTY OF MECHANICAL ENGINEERING

MUNI MED

Investigation of Mechanisms Underlying Stiffness Change in Prostate Cancer Cells:

From Tailored Experiments to Computational Simulations

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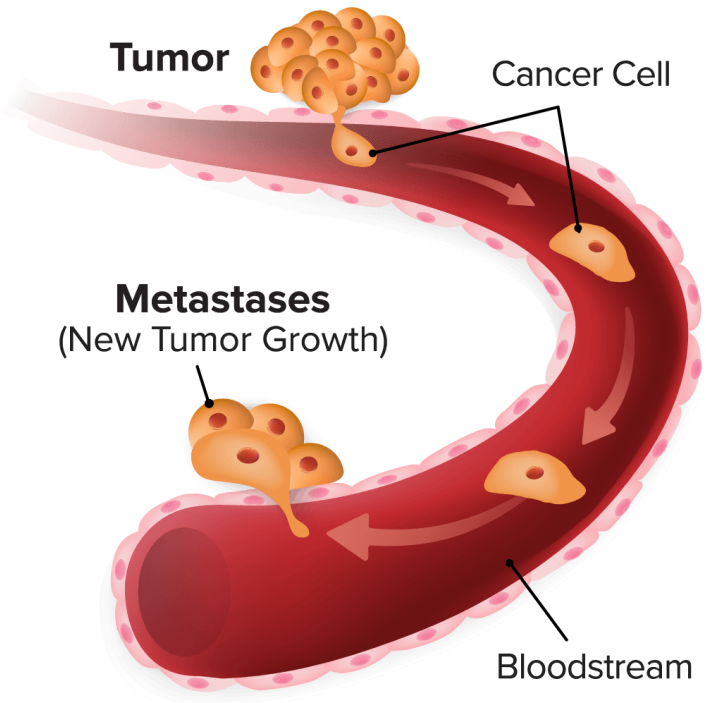
INTRODUCTION:
CELLULAR STIFNESS IN PROSTATE CANCER CELLS

- Primary stages:
  - Increased motility = higher energy demands
  - Alterations in metabolism
  - Decreased cellular stifness

- Secondary stages:
  - Stiffness increase

→ EXPLAINED BY INNER REORGANIZATION?



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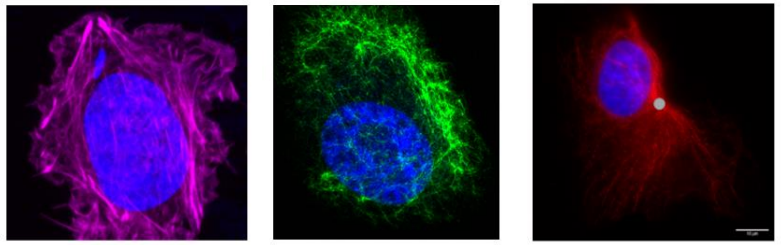
METHODS

Proteomics

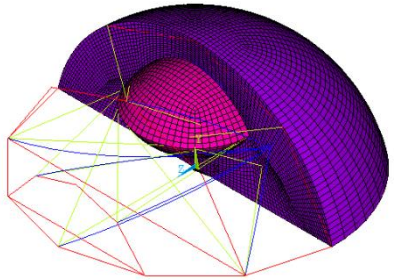
determining individual cytoskeletal proteins content



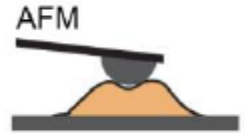
Confocal microscopy

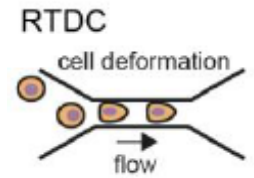
characteristic dimensions of cell lines assessment

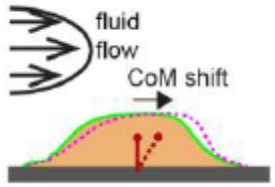
Finite Element Model



Experimental stiffness measurement

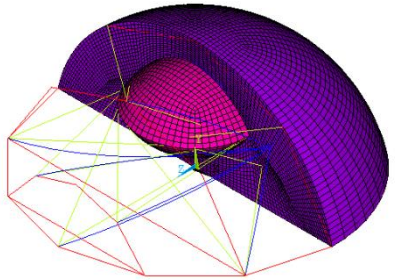






SIZE & SHAPE (mean)

	22Rv1	22Rv1 (Zn res)	PC-3	PC-3 (Zn res)
Cell radius [μm]	19.62	21.39	27.79	35.39
Cell height [μm]	8.99	9.68	9.32	9.75
Nucleus radius [μm]	11.08	11.63	13.75	14.68
Nucleus height [μm]	5.15	5.61	4.78	5.17



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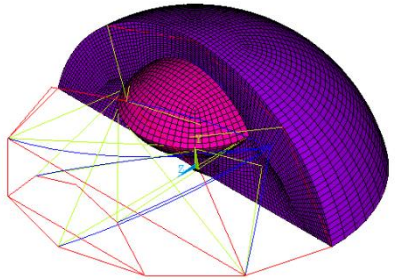
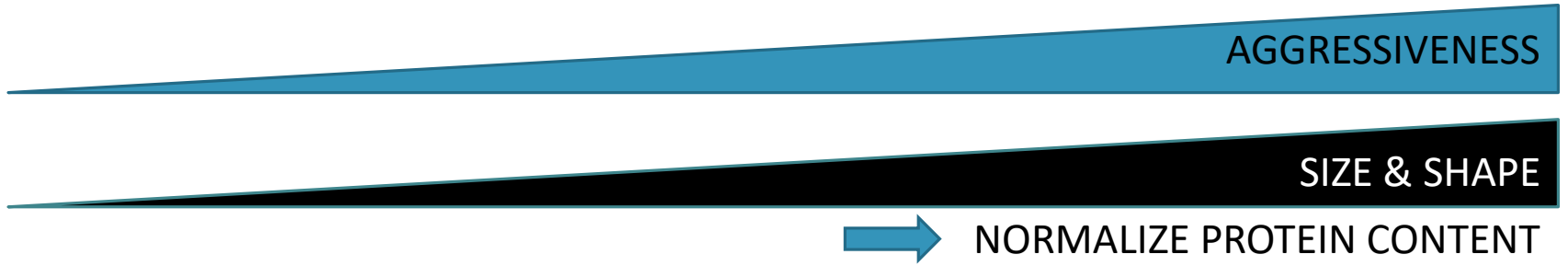


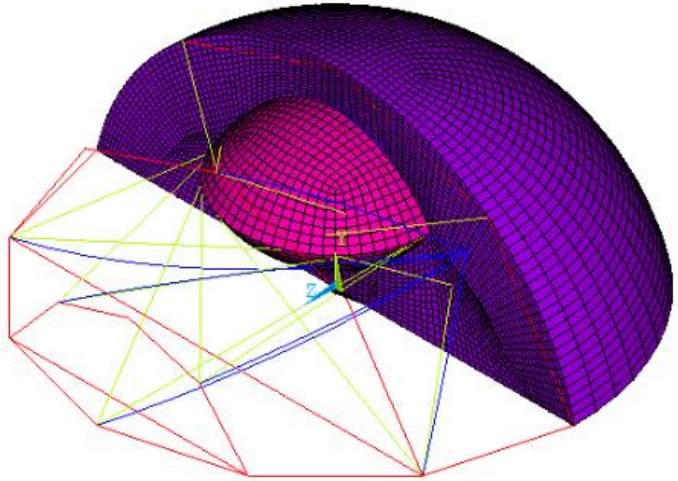


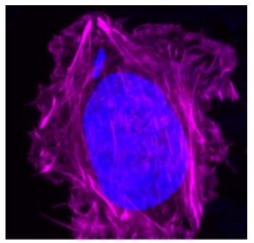
PROTEIN CONTENT (normalized)

	22Rv1	22Rv1 (Zn res)	PC-3	PC-3 (Zn res)
α/β Tubulin (MT)	100%	~100%	~100%	~100%
Vimentin (IF)	~0%	~0%	High content	High content
Actin (SF, AC)	100%	~100%	~200%	~200%

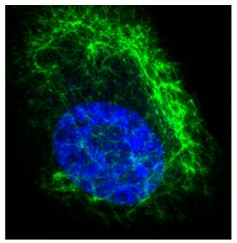
FE MODEL OF CELL (ADHERENT)

- PARAMETRIZED hybrid computational model
  - Continuum (Neo-Hookean solid): nucleus, cytoplasm, actin cortex and membrane
  - Discrete elements: cytoskeleton with pre-stressed ABs and wavy IFs

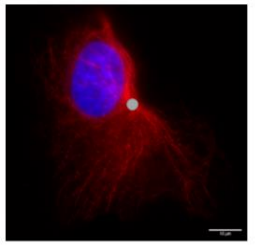




AB (SF)



IF



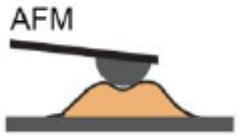
MT

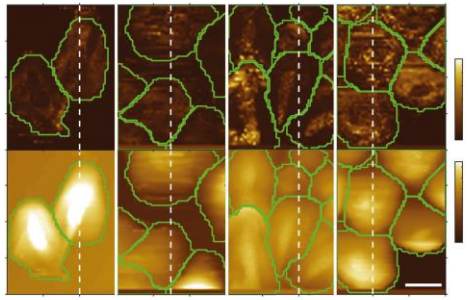
------ Actin bundles (ABs)
------ Intermediate filaments (IFs)
------ Microtubules (MTs)

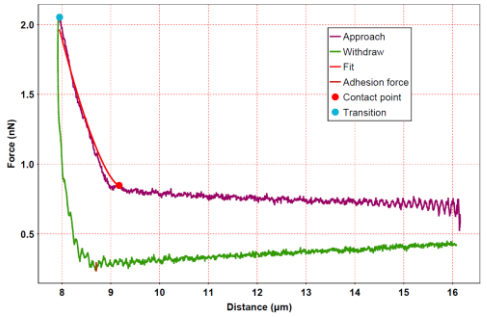
------ Nucleus
------ Cytoplasm

STIFFNESS MEASUREMENT METHODS

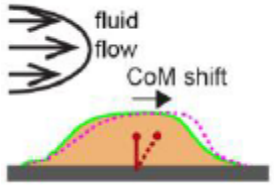
- Local stifness mapping: AFM

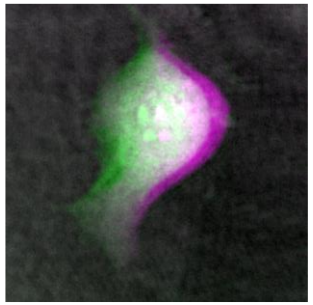


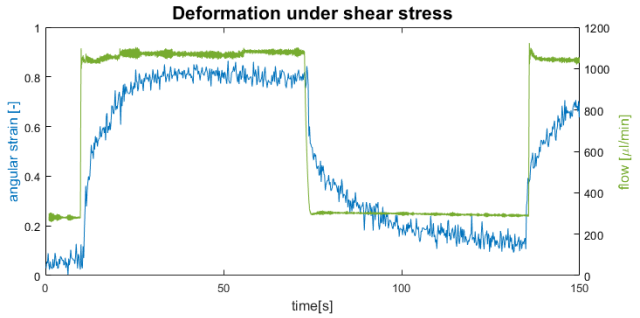


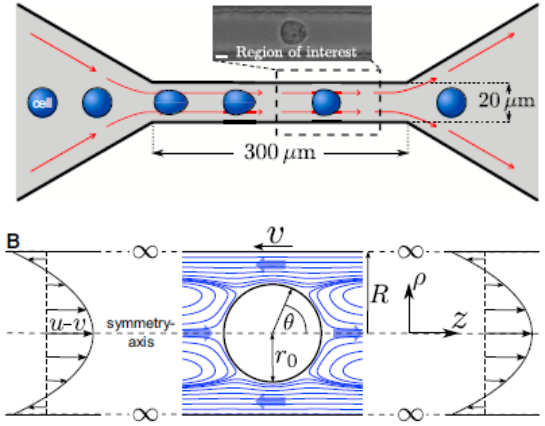


- Global level: Shear & Real-time Deformability Cytometry (RT-DC)









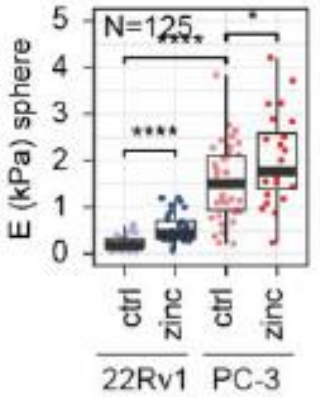
RT-DC setup from Mietke, 2015

CELLULAR STIFNESS IN PROSTATE CANCER CELLS

22Rv1 have almost no vimentin compared to PC-3 cell line

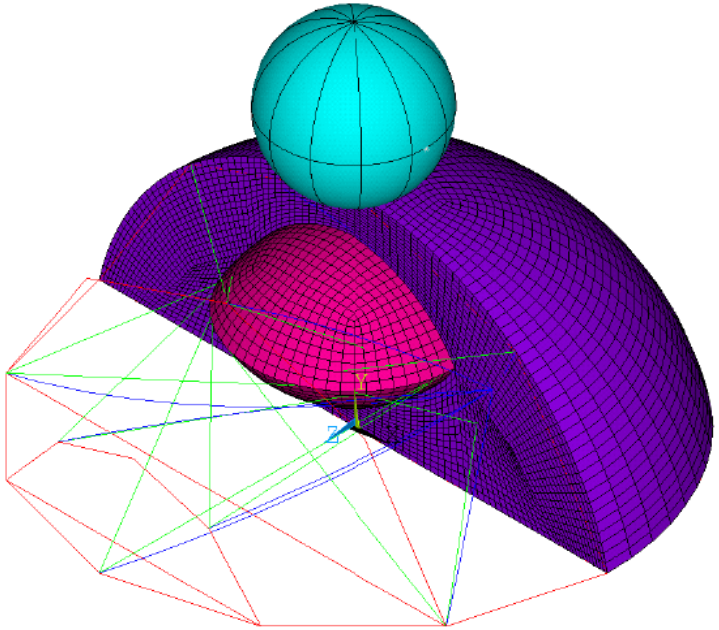
PC-3 cells have double the content of actin proteins

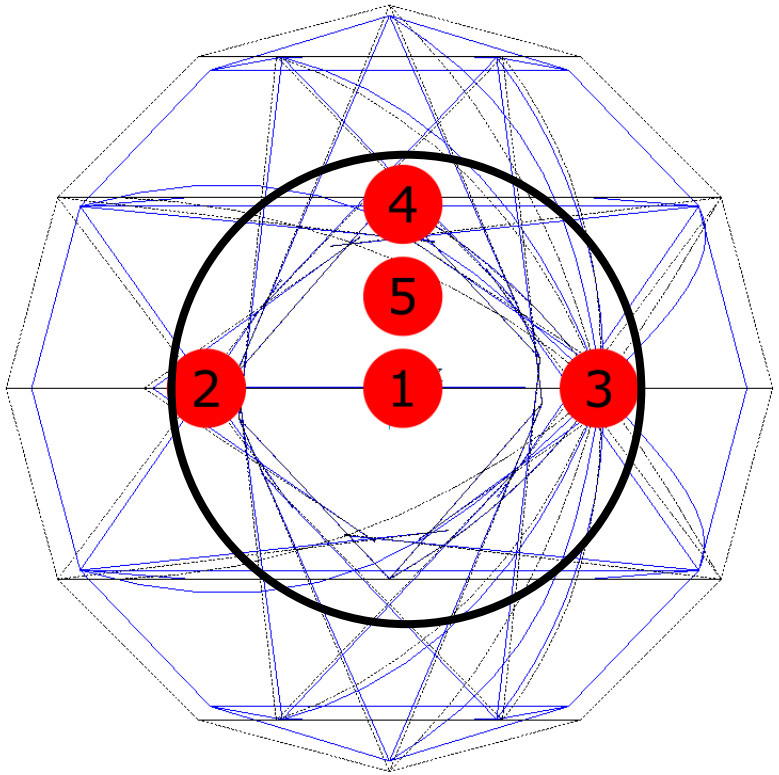
?



AFM setup

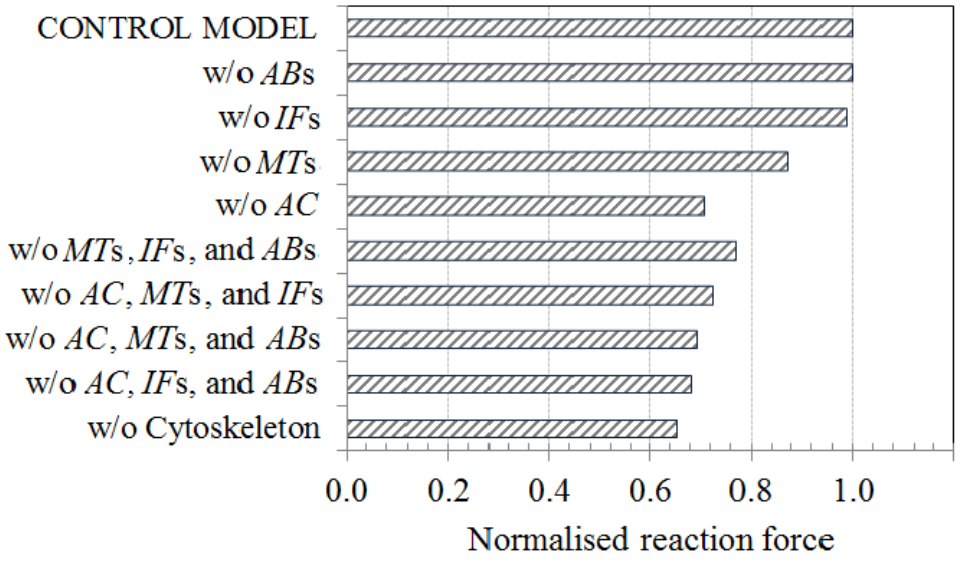
1. Centered above nucleus (apex)
2. Above centrosome
3. Symetrically with 2. (in the cytoplasm)
4. At a receptor
5. Between apex and receptor

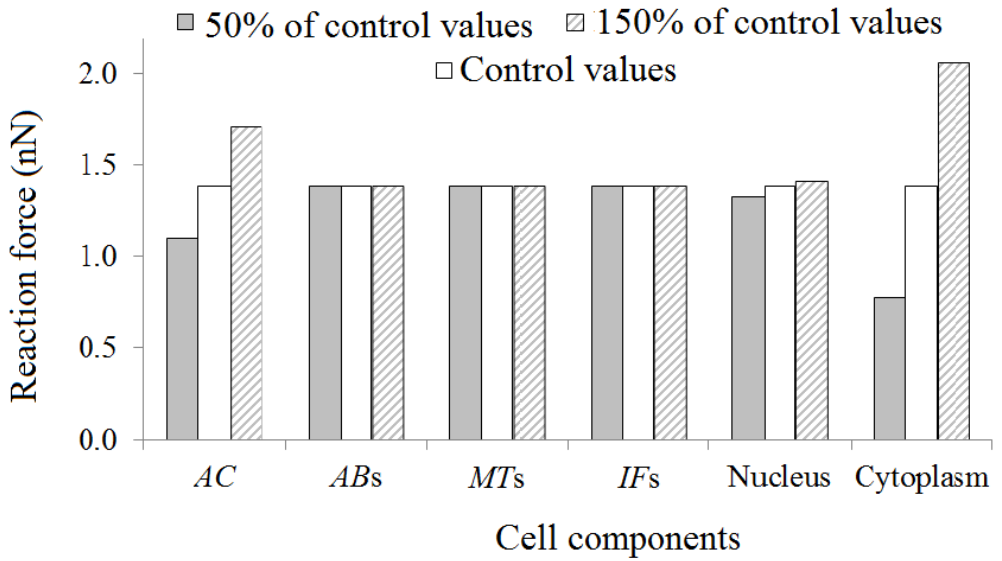




------ **Actin bundles (ABs)**
------ **Intermediate filaments (IFs)**
------ **Microtubules (MTs)**
------ **Nucleus**
------ **Cytoplasm**
------ **Indentor**

CYTOSKELETAL CONTRIBUTION TO CELL STIFFNESS





YD Bansod, 2016

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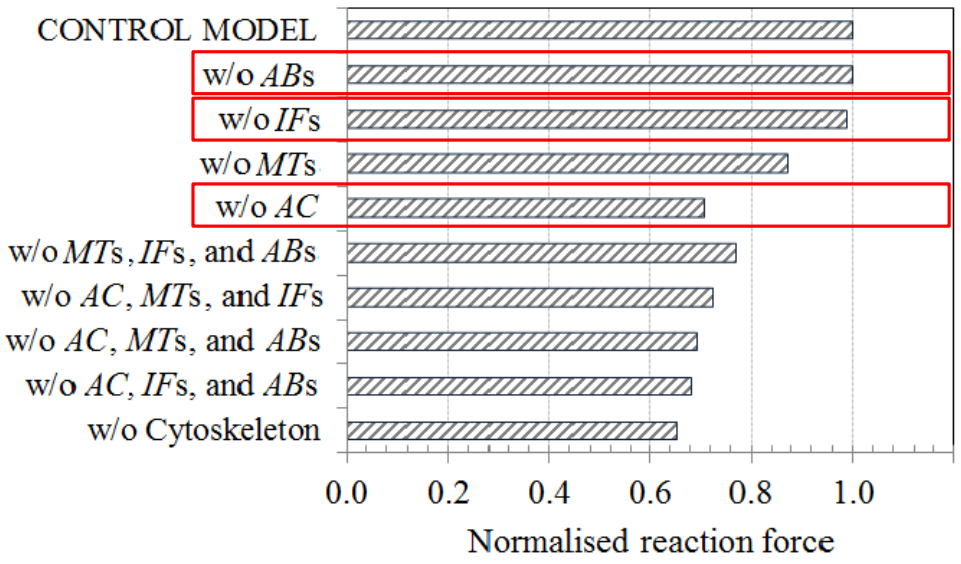
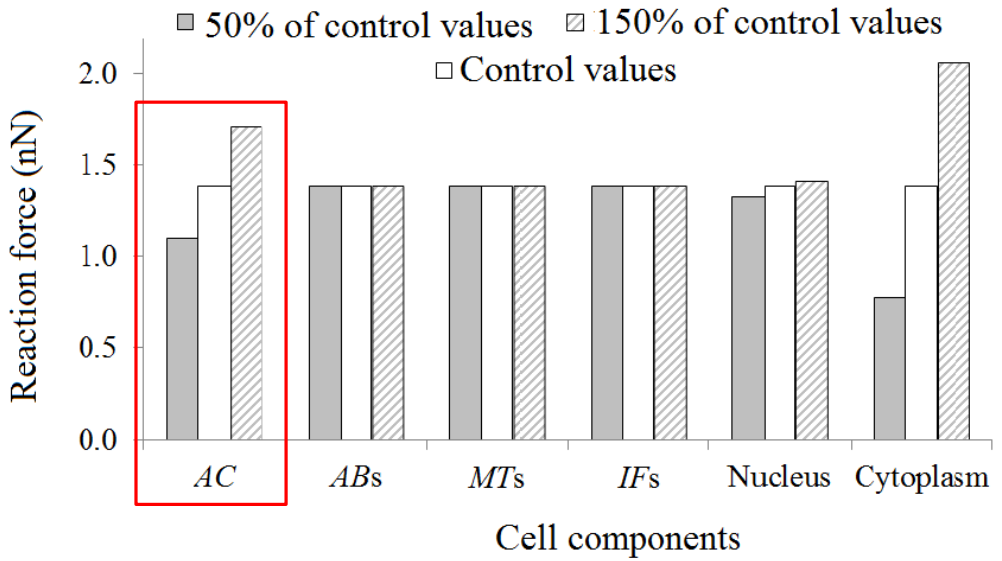


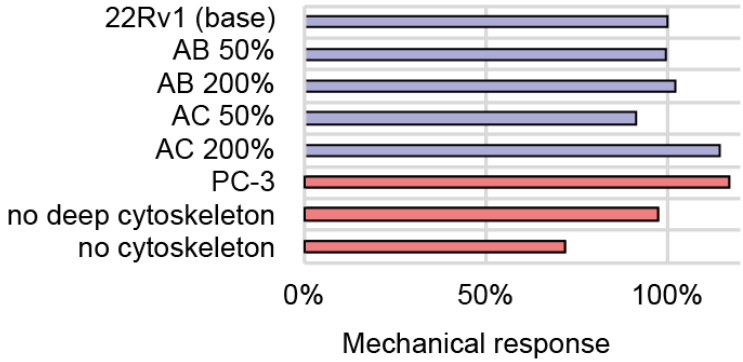


YD Bansod, 2016

RESULTS & DISCUSSION

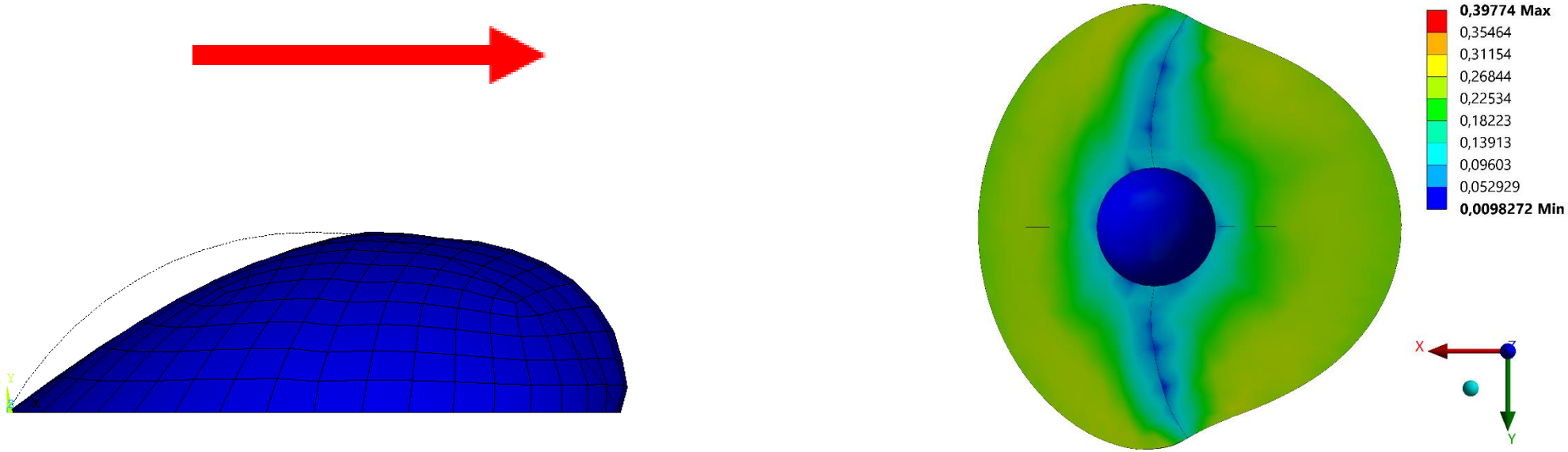
- AFM: vimentin and „deep cytoskeleton“ role only minor
  - Actin cortex (AC) the most prominent among the organelles
- Shear & RT-DC: more pronounced response to cytoskeleton modifications (at the extremity of experimental boundary conditions > 20%)

- The ability of the FE model to mimic various experimental setups (easily adaptible)
- Allows for parametric studies



SHEAR & RT-DC

- Submodelling: boundary conditions from continuous problem transferred to hybrid model
- Strain distribution throughout all of the cytoplasm > 20%



LIMITATIONS of FEM simulations

- ✖ Active cytoskeleton response (remodelling & contractility, etc.)
  - → non-linear CSK properties
- ✖ Viscoelastic behavior of the cell → Vicar, 2022
- ✖ Compressibility of cytoplasm
- ✖ Adhesion forces (AFM evaluated by DMT & JKR models)

To clarify the full extent of the stiffness change,
other organelles may play crucial role

RESEARCH TEAM

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FEM formulated by
Y. D. Bansod & V. V. S. V. Jakka

Experimental work
Members of Masarik lab

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Thank you for your attention!