ROLE OF CAVEOLIN IN CELLULAR TRACTION FORCE GENERATION

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Some terminologies….

Mechanotransduction -

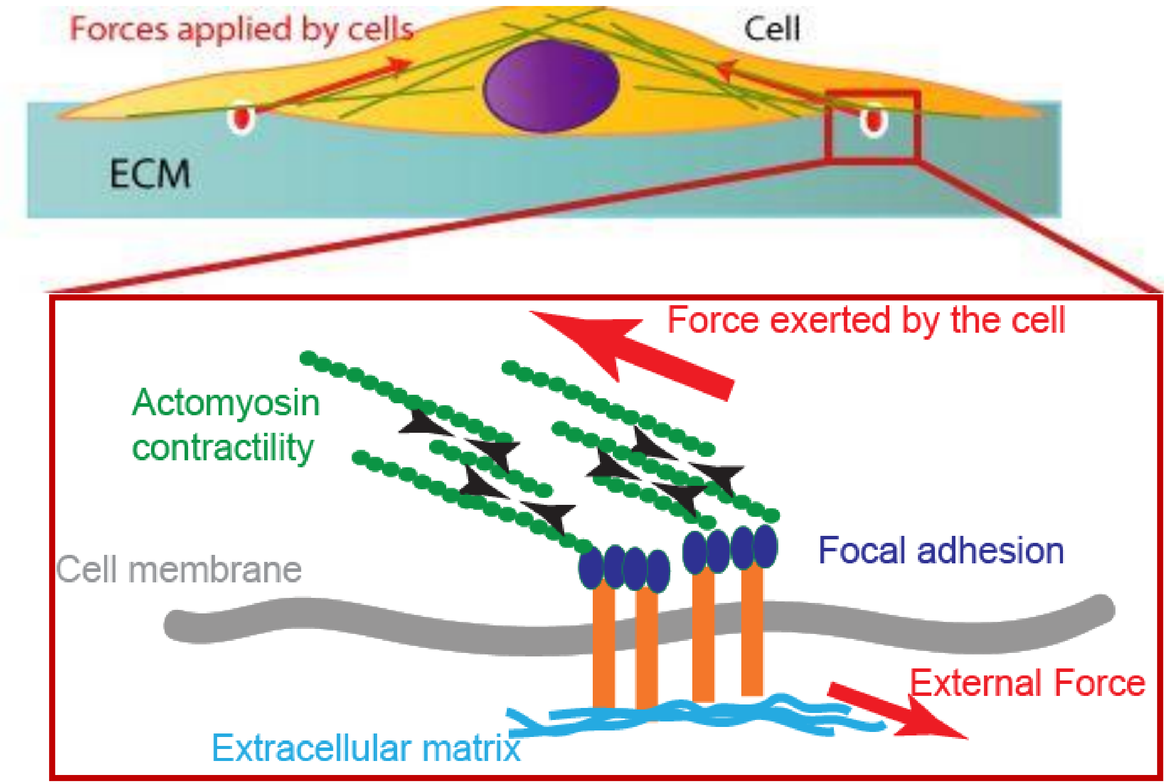
- The process by which cells **sense mechanical stimuli** and convert into chemical or electrical signal
- Occur at cell surface , mostly at **FA sites**
- mediated majorly through **mechanosensitive proteins** like focal adhesion proteins

Focal adhesions-

- Sites at which **cells form attachment to substrate**
- Macromolecular assemblies through which **mechanical force and regulatory signals are transmitted** between the ECM & cell

Traction force –

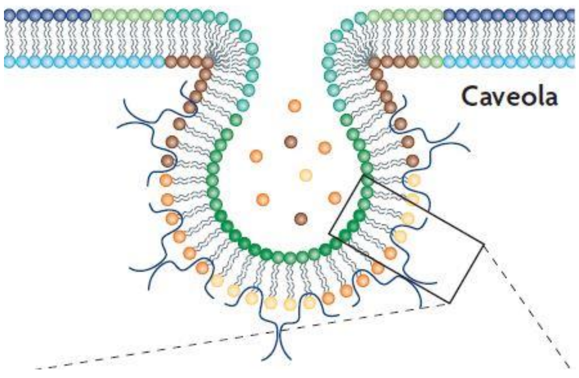
- Contractile forces exerted by cell on substrate through FA and actomyosin cytoskeleton
- local force per unit area that is imposed on the microenvironment



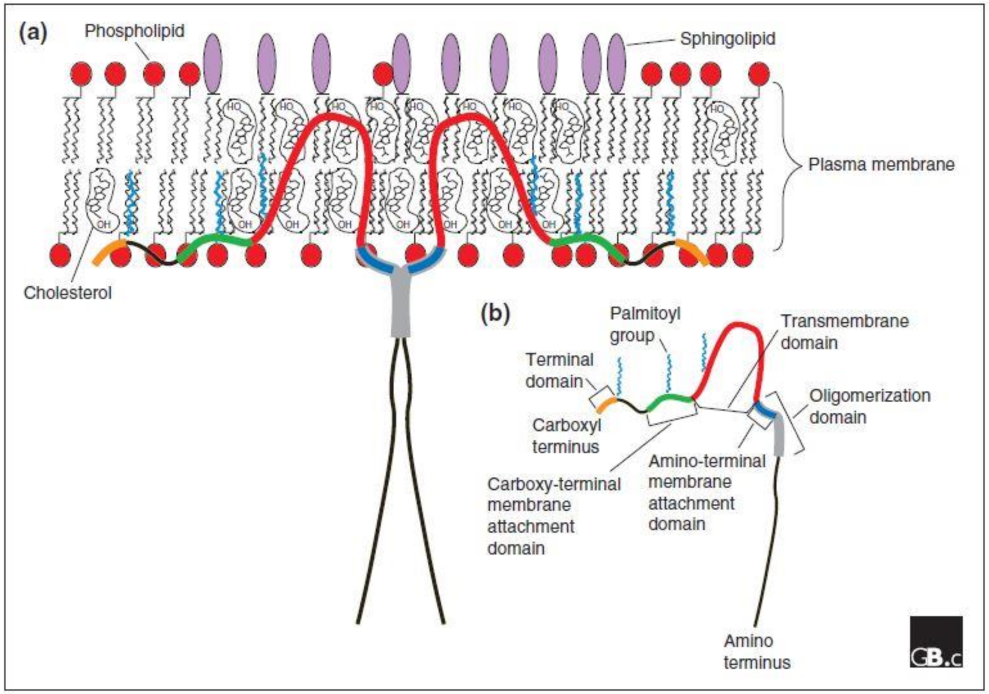
Caveolin

Caveolin – transmembrane protein involved in caveolae formation and maintenance

Caveolae - Latin for “little caves”
invaginations on cell surface and is involved in transcytosis, compartmentalization and concentrating signaling molecules.



“The multiple faces of caveolae”, Nature

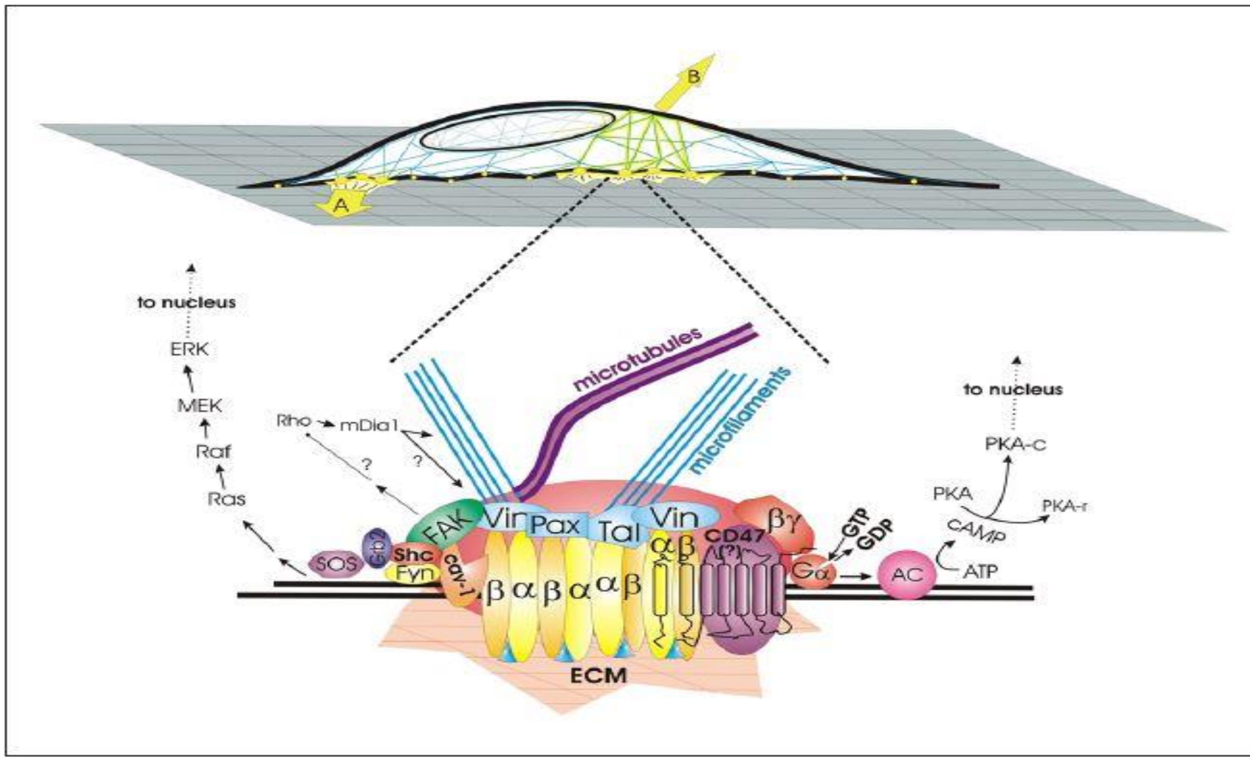


“The caveolin proteins”, *Genome Biology*

Recent findings…

- **Increased flow** or shear stress induced **phosphorylation of cell surface proteins** & their **localization in caveolae**. Activated Ras- Raf- MAPK signaling cascade

- **Caveolin is associated** with focal adhesion proteins such as **integrins**, which stimulates **caveolin-1 phosphorylation** and recruitment of Csk to mediate actin reorganization



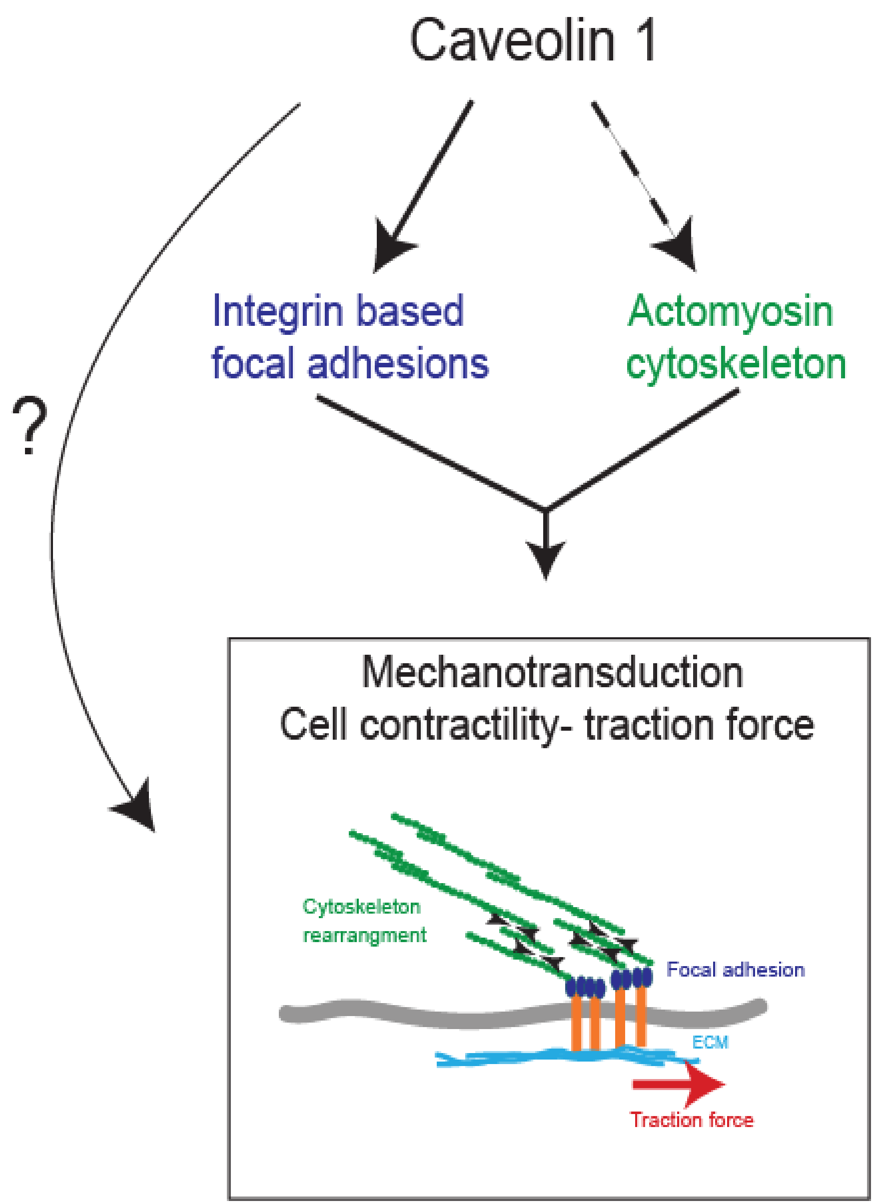
“Mechanotransduction: All Signals Point to Cytoskeleton, Matrix, and Integrins” *Science's STKE,* 2002

Recent findings…

- Caveolin KO mice has defects in remodeling blood vessel

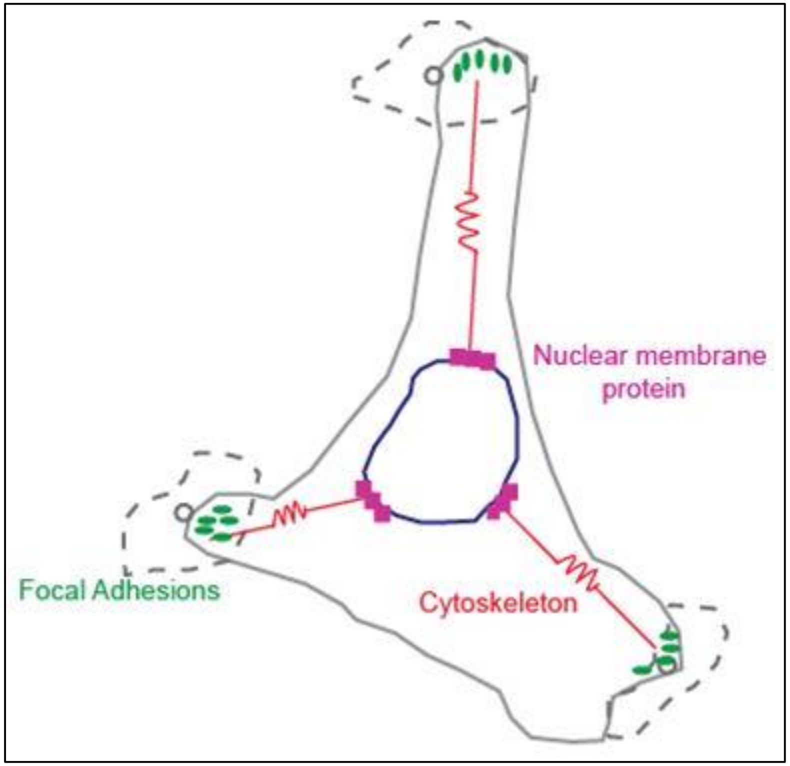
-Decrease in blood flow did not reduce lumen diameter

- **Reduction of caveolin-1 expression** disrupts integrin interaction with Src kinase and **induces loss of focal adhesion sites**, ligand-induced FAK phosphorylation and adhesion.



Caveolin 1
?
Integrin based
focal adhesions
Actomyosin
cytoskeleton
Mechanotransduction
Cell contractility- traction force
Cytoskeleton
rearrangment
Focal adhesion
ECM
Traction force

Influence of caveolin in regulating cell contractility

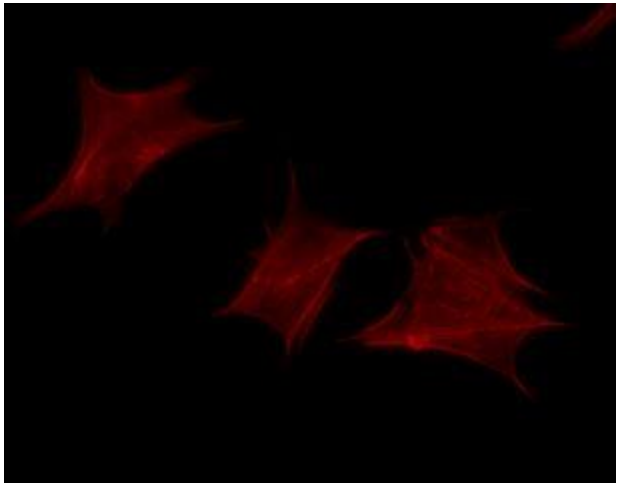


Comparison of **WT** and ***Cav1*** **KO** MEF cells (mouse embryonic fibroblasts) on 10kPa and 40kPa substrates-

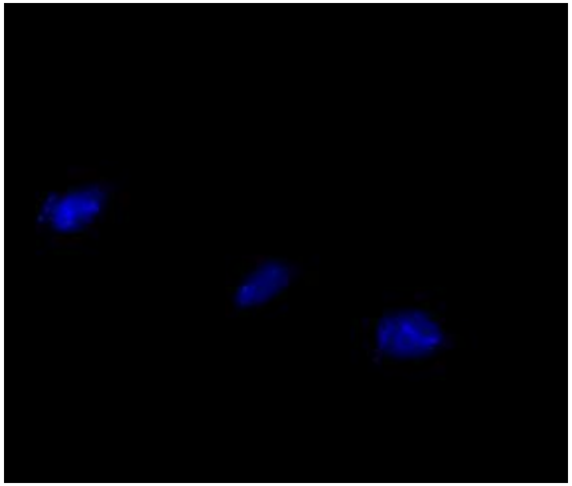
- Morphology analysis
- Traction analysis

Morphology Analysis

- Seeding cells on poly-acrylamide gel
- Fixation & permeabilization
- Staining of actin with rhodamine phalloidin
- Staining of nucleus with DAPI

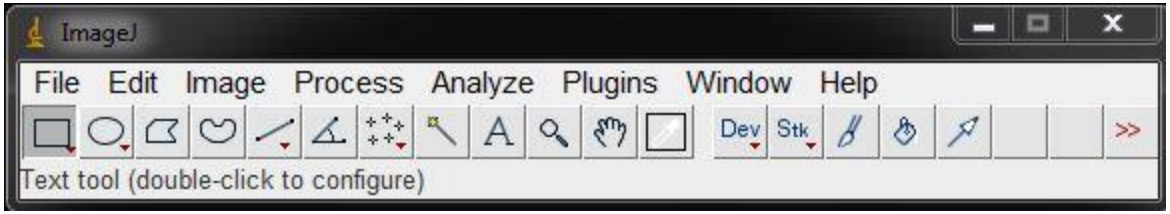


Rhodamine phalloidin stain
actin

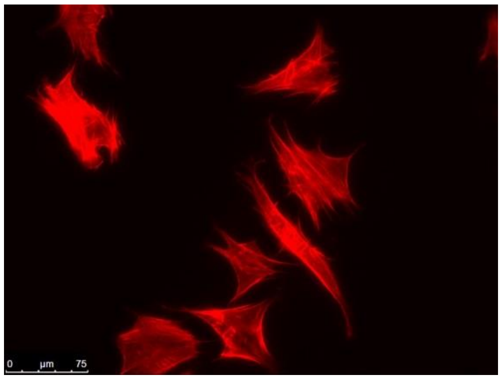


DAPI stain
nucleus

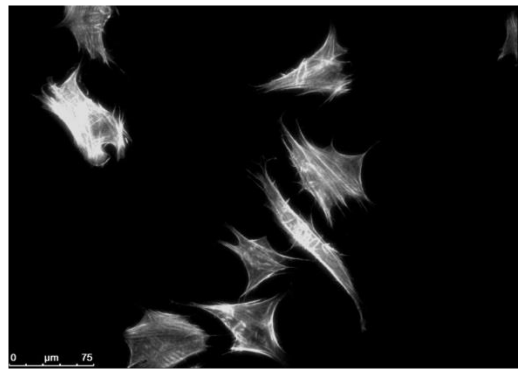
Courtesy – Nidhi Sinha



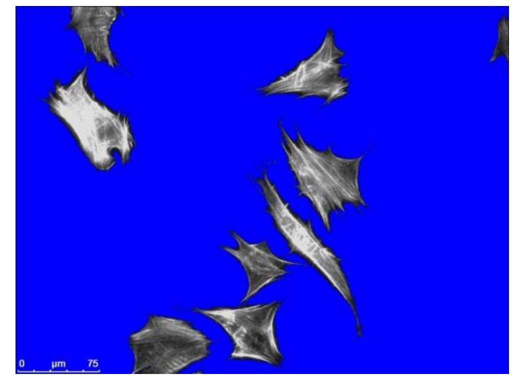
cell



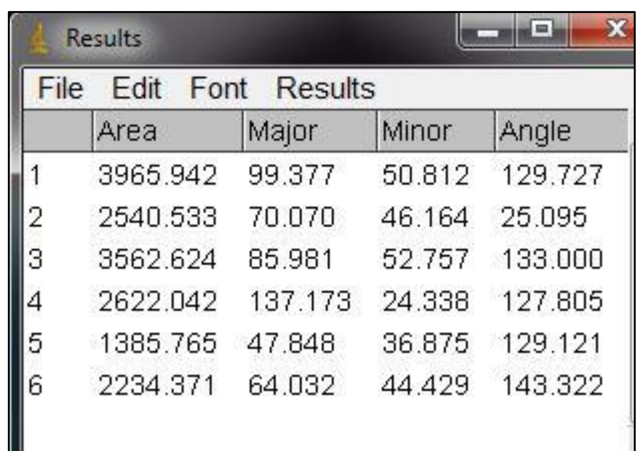
8 bit image



Threshold



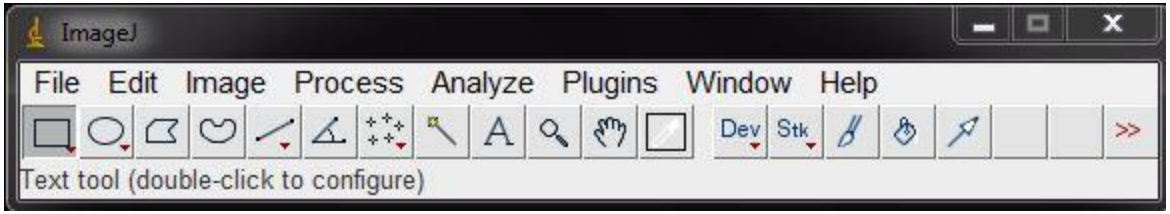
Result



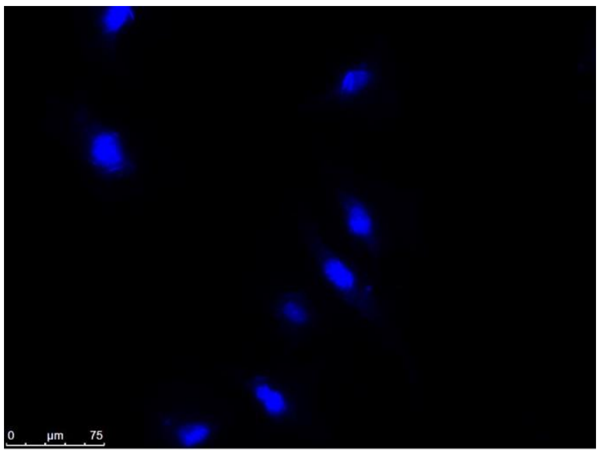
Results

File Edit Font Results

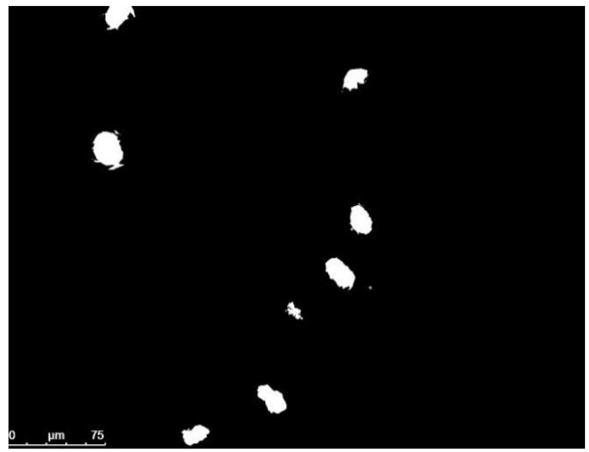
	Area	Major	Minor	Angle
1	3965.942	99.377	50.812	129.727
2	2540.533	70.070	46.164	25.095
3	3562.624	85.981	52.757	133.000
4	2622.042	137.173	24.338	127.805
5	1385.765	47.848	36.875	129.121
6	2234.371	64.032	44.429	143.322



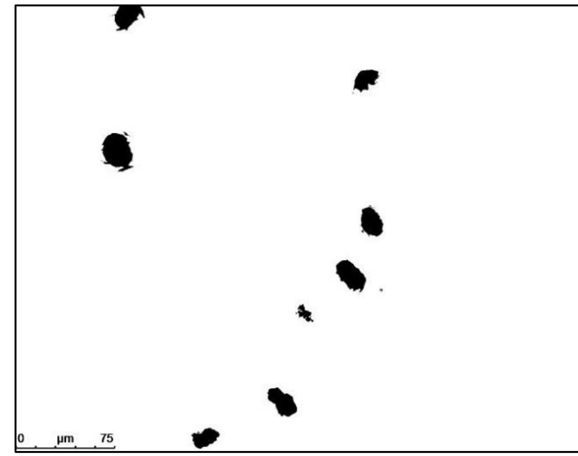
Nucleus



Threshold



Binary



Result

Results

File Edit Font Results

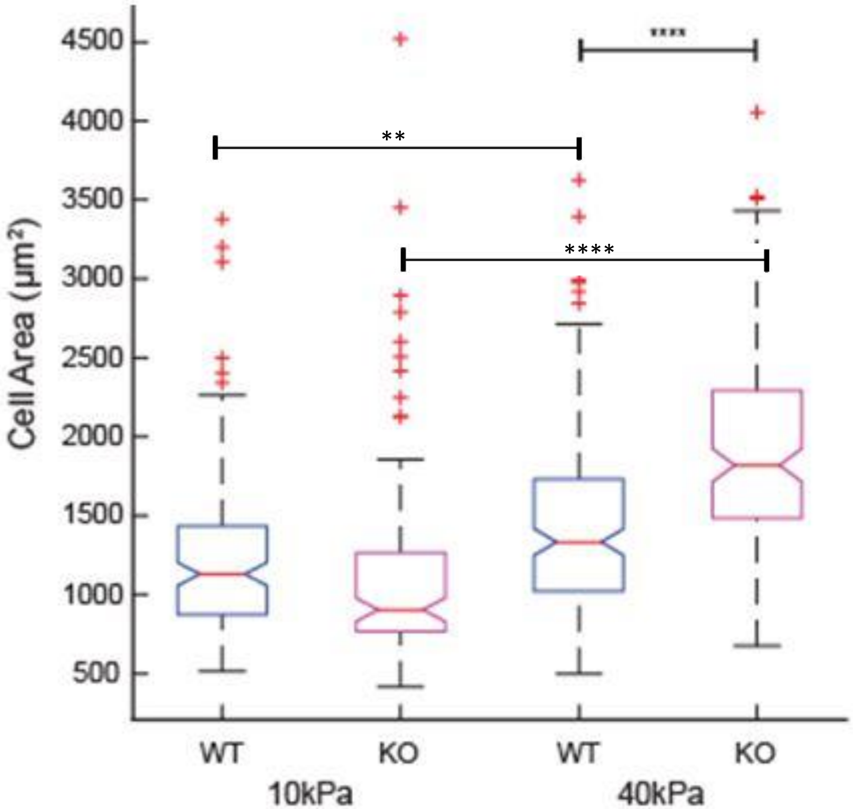
	Area	Major	Minor	Angle
1	282.587	22.957	15.673	40.775
2	180.885	19.682	11.702	37.021
3	421.184	26.297	20.392	113.440
4	254.466	22.036	14.703	114.256
5	316.311	26.495	15.201	134.540
6	282.587	25.848	13.920	135.256
7	177.396	20.254	11.152	26.930
8	82.355	70.813	1.481	0.024

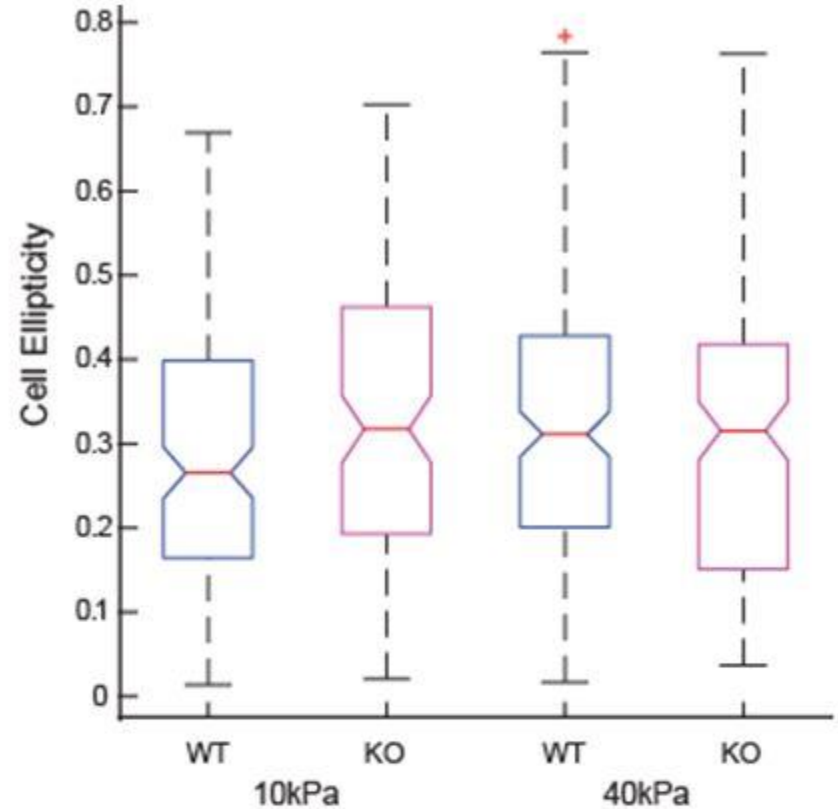
Cell Morphology- statistical analysis

- ANOVA with Bonferroni multicompare test
- Confidence interval > 95 %
- Box Plots

n > 100

Cell



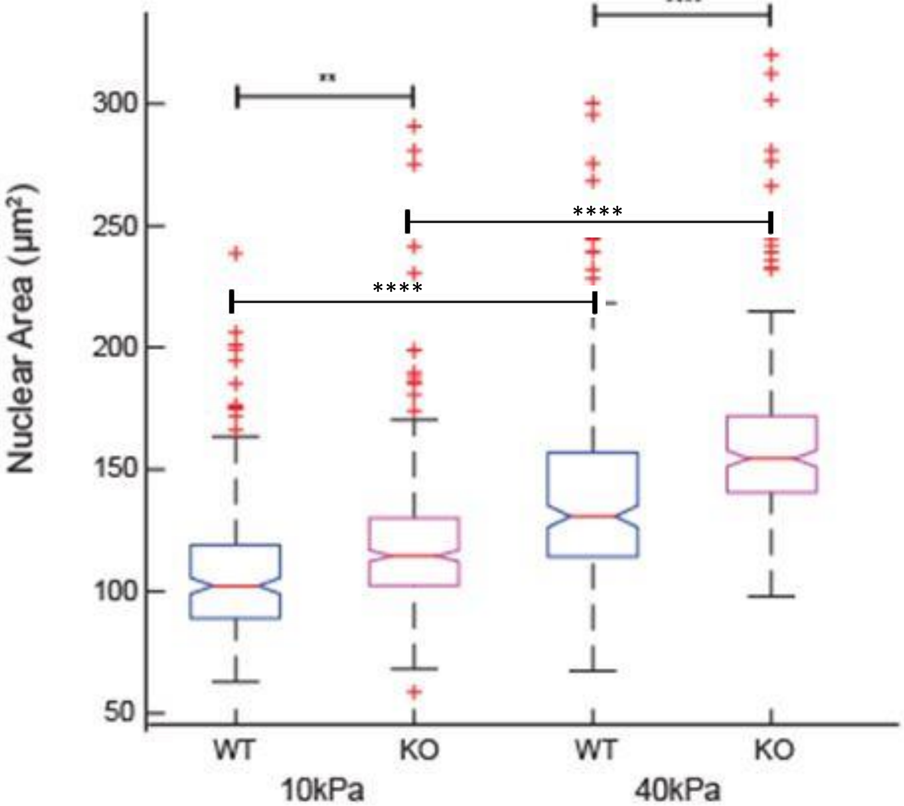


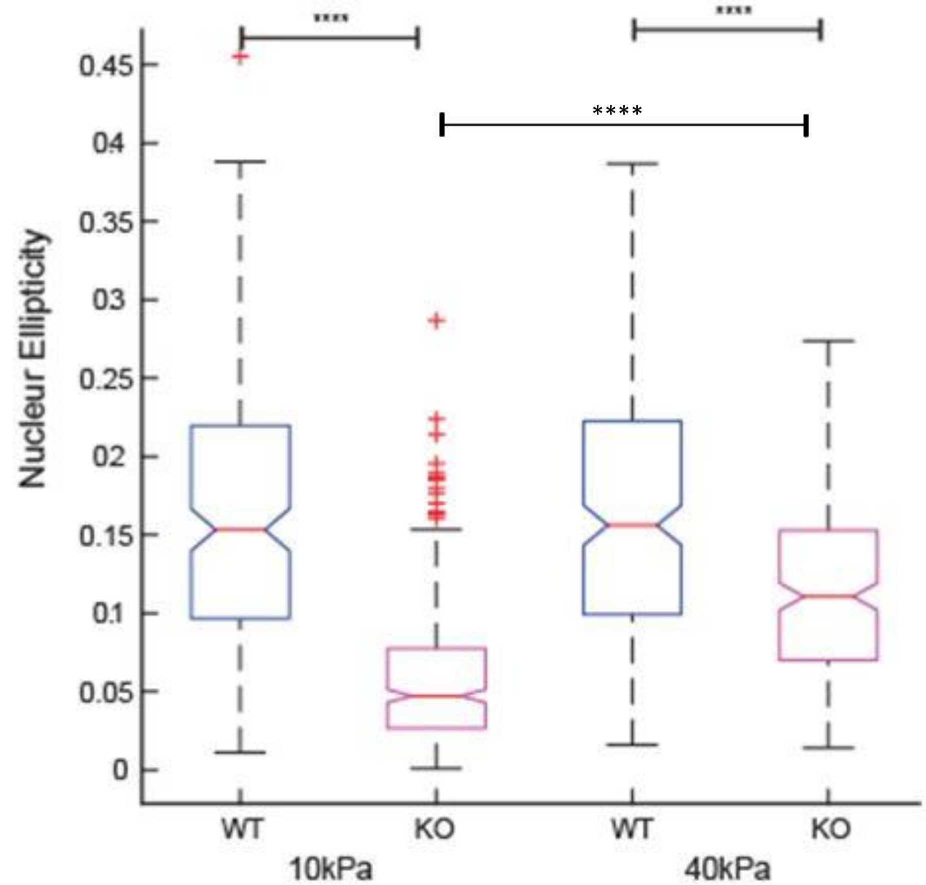
Significant difference in
- ✓ WT 10 kPA & WT 40 kPa
- ✓ KO 10 kPa & KO 40 kPa
- ✓ WT 40 kPa & KO 40 kPa

NO Significant difference

n > 200

Nucleus





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- ✓ WT 10 kPa & KO 10 kPa
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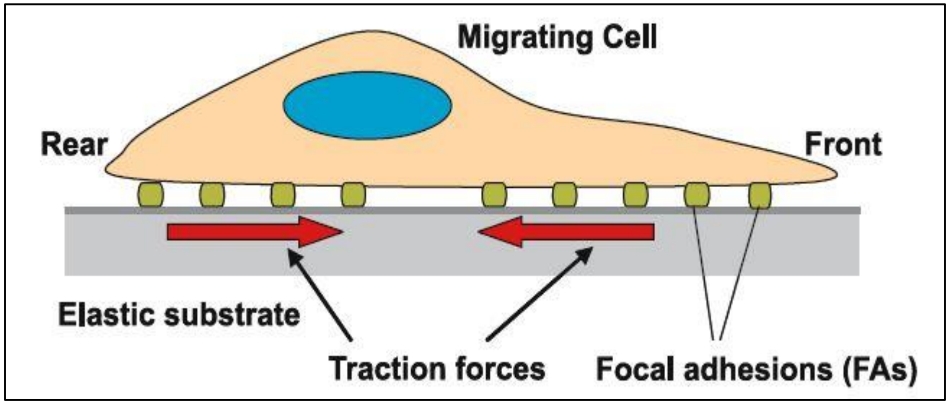
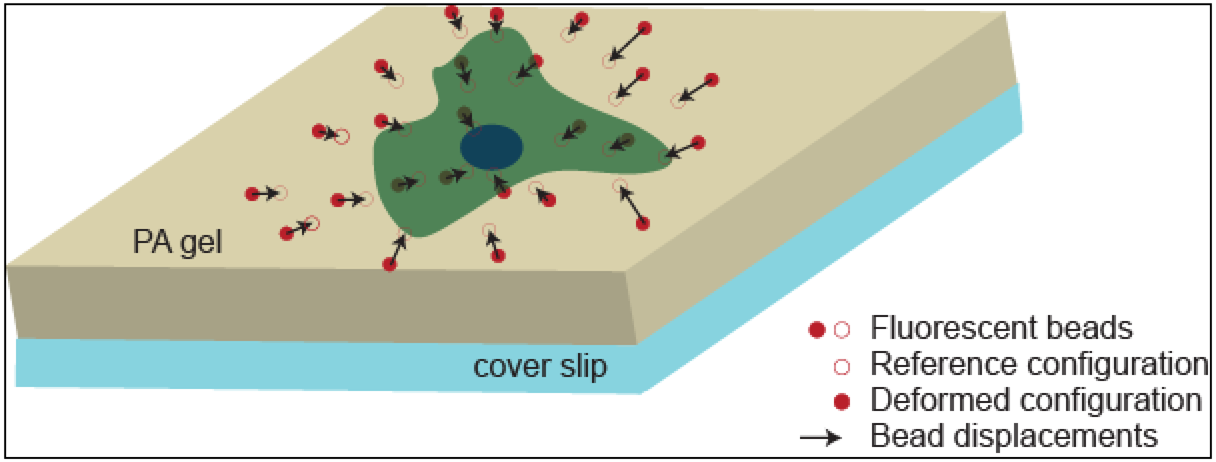
Traction Force Microscopy

Technique which allows us to measure the forces exerted by cells on its substrate



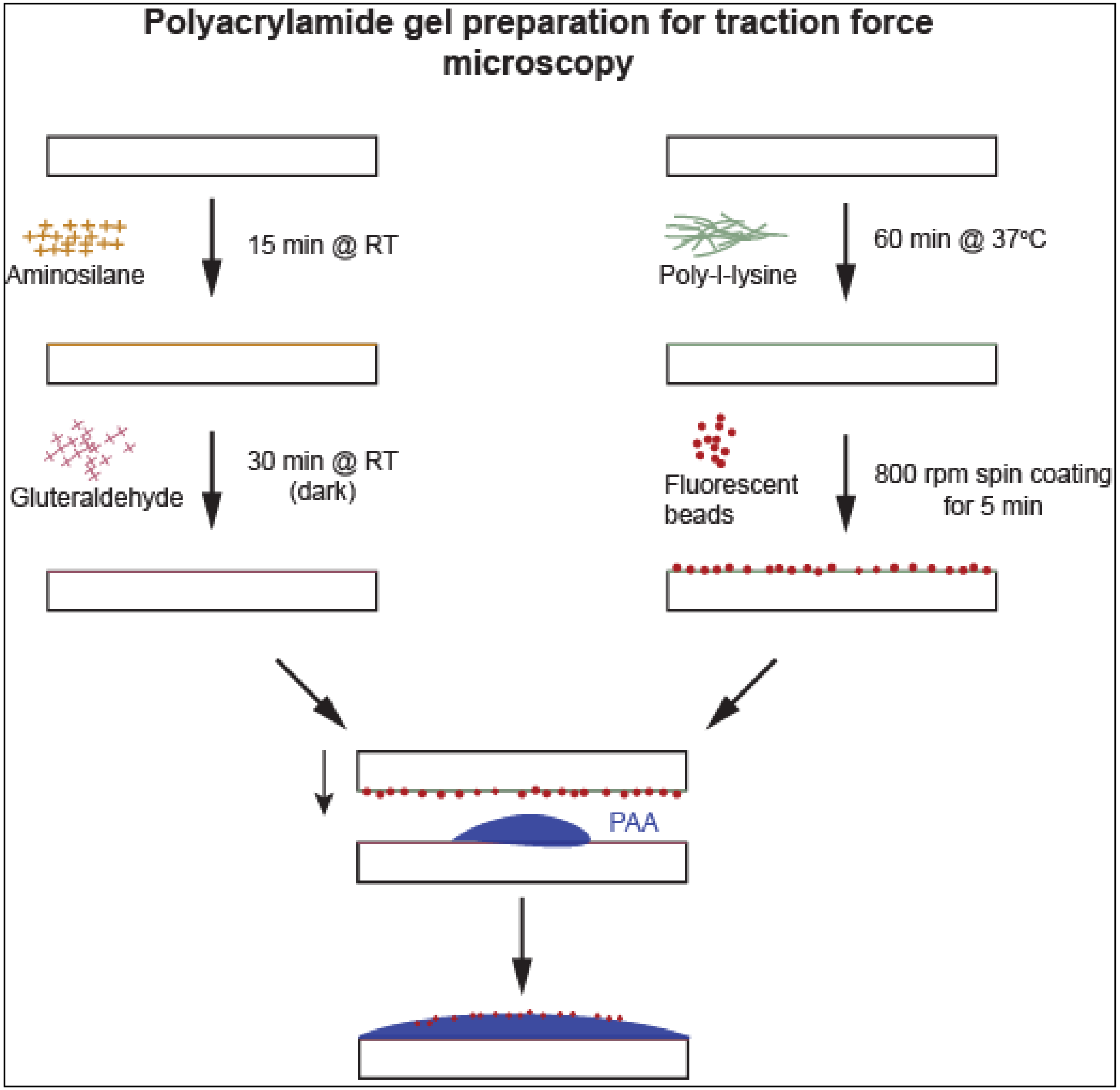
Fluorescent beads



James H.-C.Wang, Jeen-Shang Lin, and Zhao-Chun Yang ,*Cell Traction Force Microscopy*

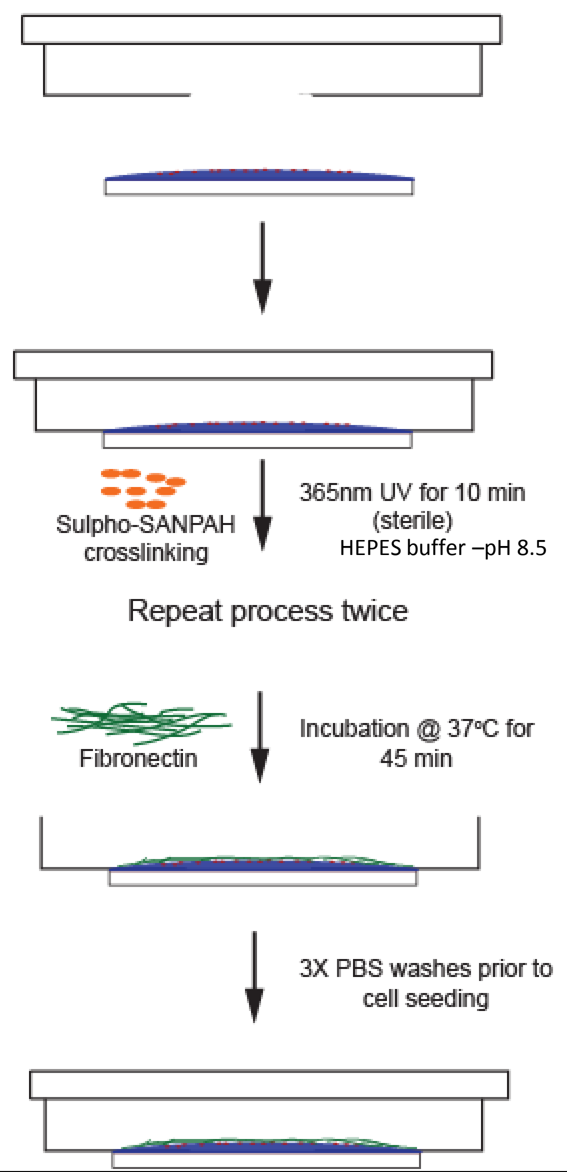
Steps involved in traction force microscopy

1. Gel fabrication & cell seeding
2. Image of beads in stressed condition & phase contrast image of cell
3. Trypsinization to detach cells
4. Image of beads in relaxed state
5. Calculation of bead displacement & traction

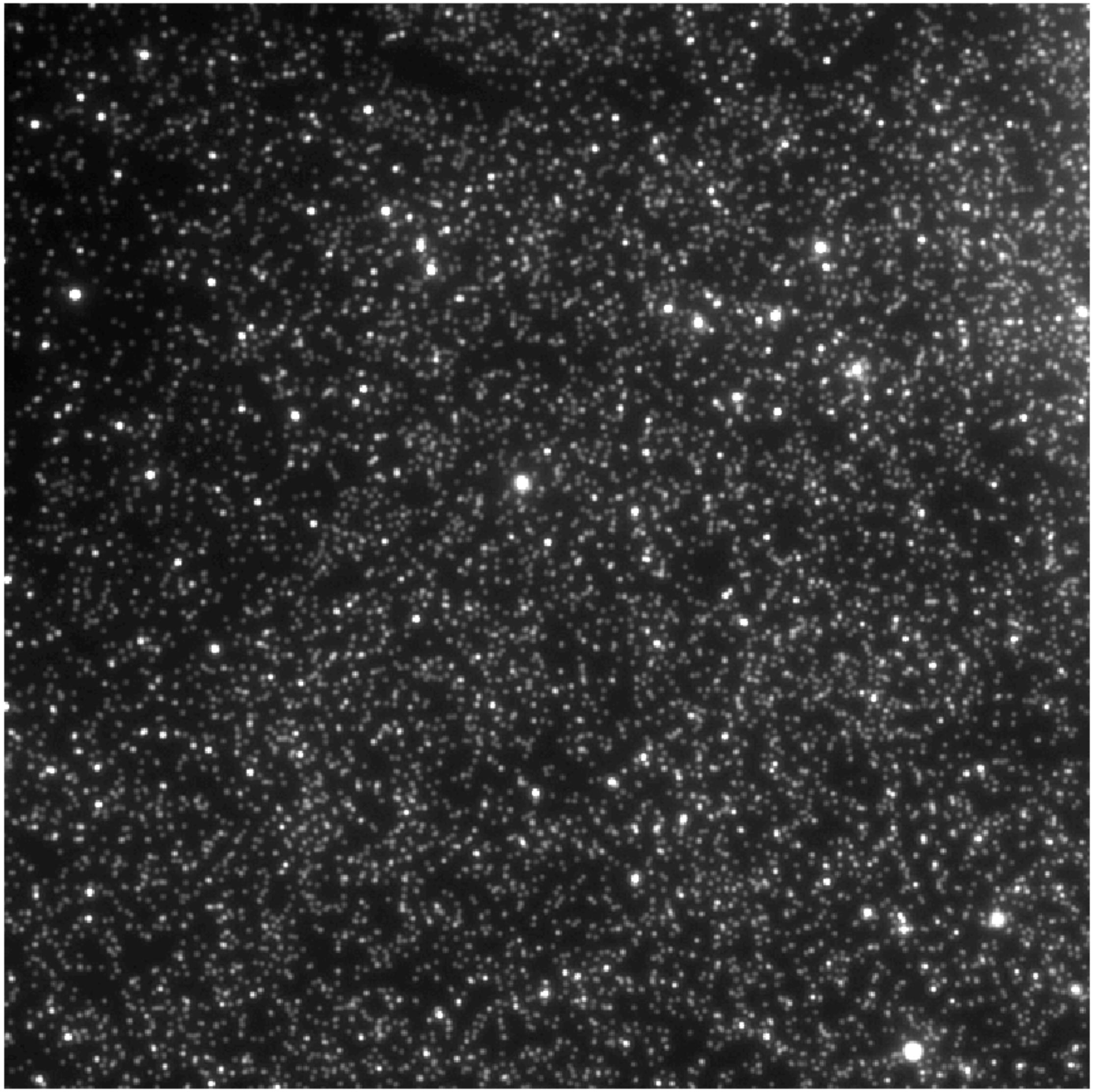


Polyacrylamide gel preparation for traction force microscopy
Aminosilane
15 min @ RT
Poly-l-lysine
60 min @ 37°C
Gluteraldehyde
30 min @ RT (dark)
Fluorescent beads
800 rpm spin coating for 5 min
PAA

Sulpho-SANPAH treatment for ECM coating of polyacrylamide gel

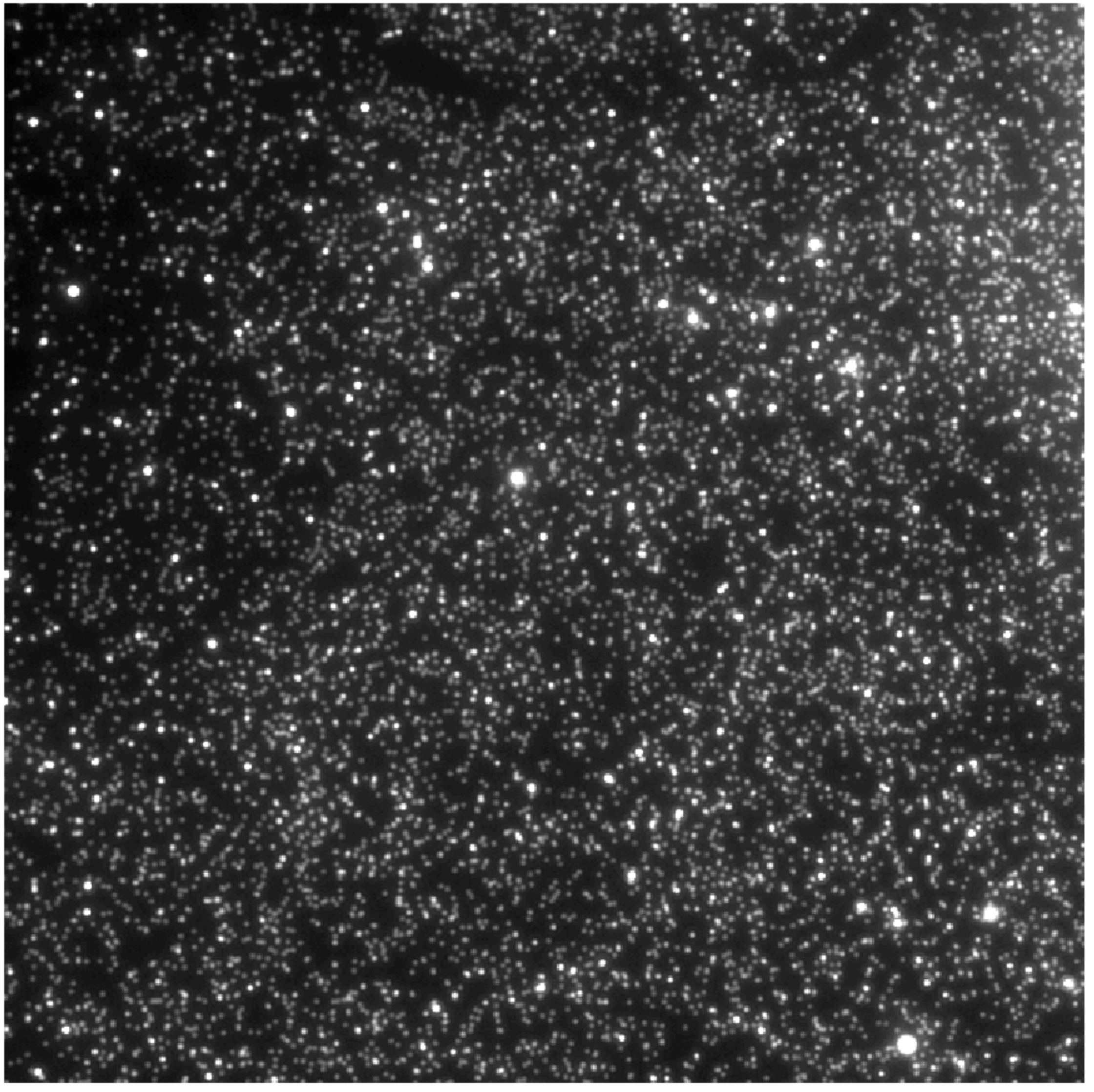


Before Trypsin



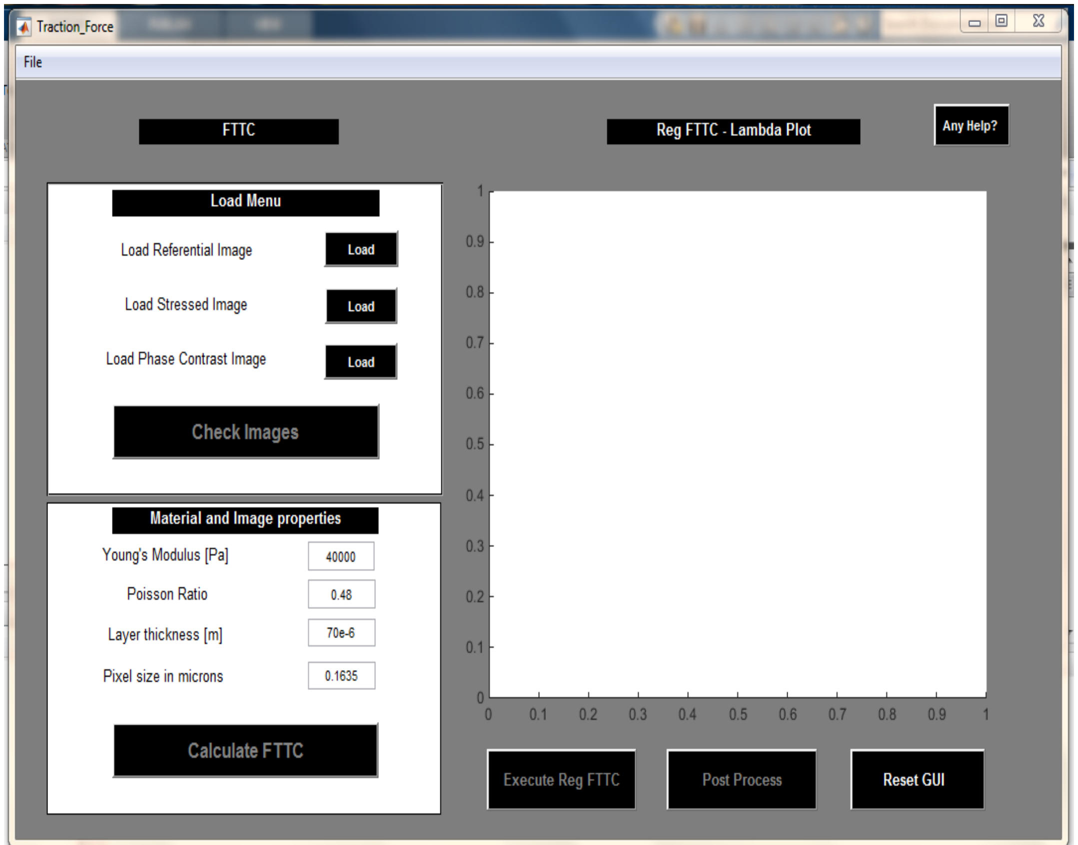
Fluorescent image of beads in Stressed Condition

After Trypsin

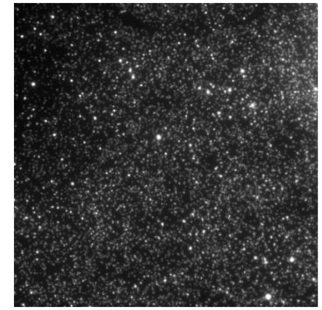


Fluorescent image of beads in Relaxed Condition

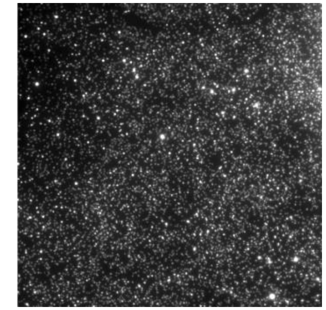
Traction force microscopy GUI



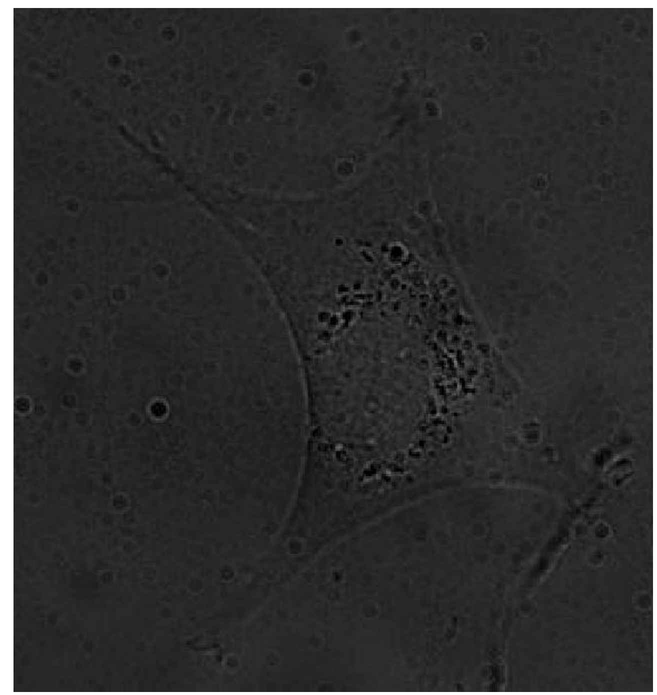
Pre



Post



Cell



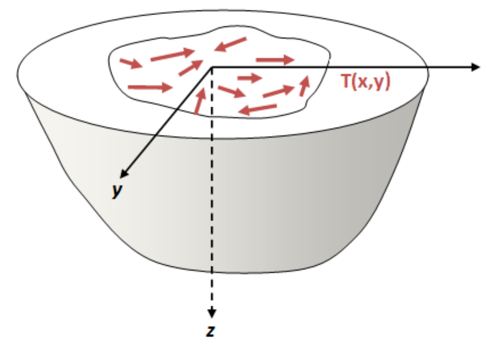
Regularized FTTC code modified by Ashwin S.

Fourier Transform Traction Cytometry

Image → (DIC — Digital Image correlation) → Displacements → (FTTC) → Force

$$G.T = u$$ .....Bousinessq

$$T = G^{-1}(u)$$ ... inverse problem



Half-space Model

Fourier Transformation- (Butler)

Number of unknowns reduced

Solves inverse problem

Consider tangential forces applied to free surface of semi infinite solid.

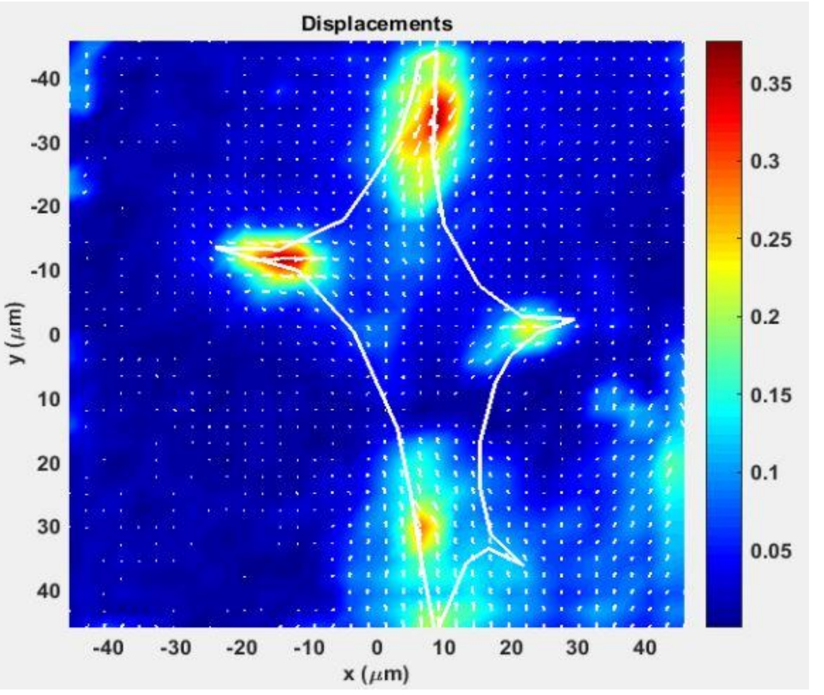
Benefits from a very fast computation time

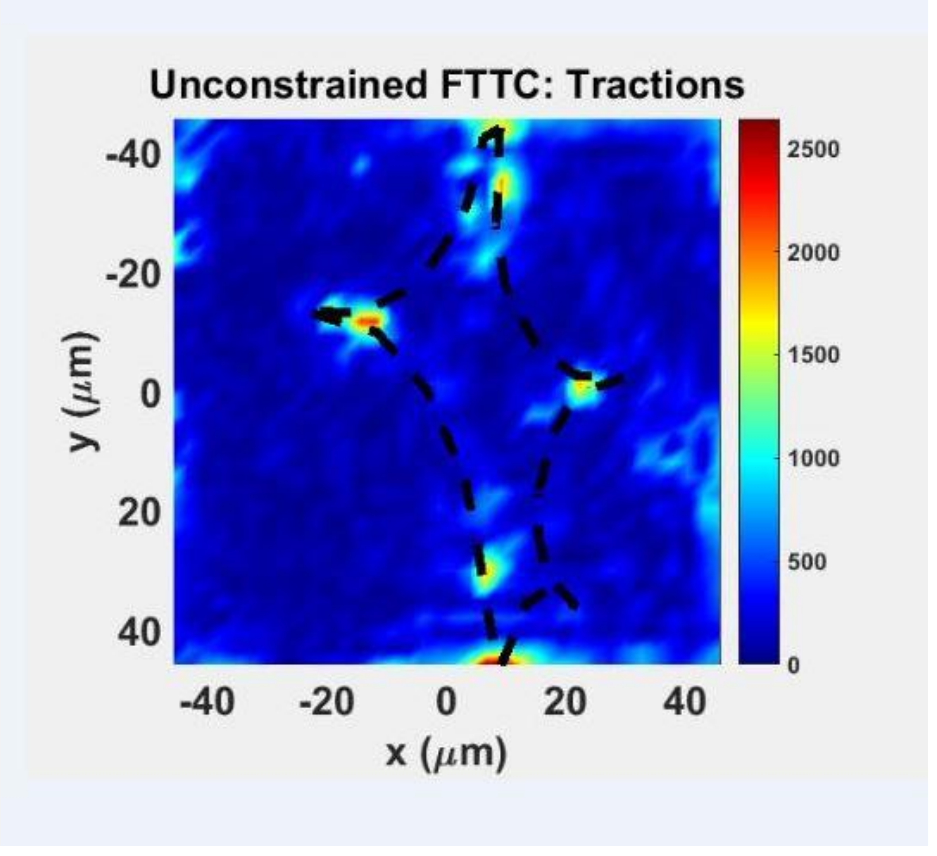
Regularization -

DIC – averages displacements - generate errors

Small errors in displacement cause amplification of error in traction

Displacements generated by FTTC code





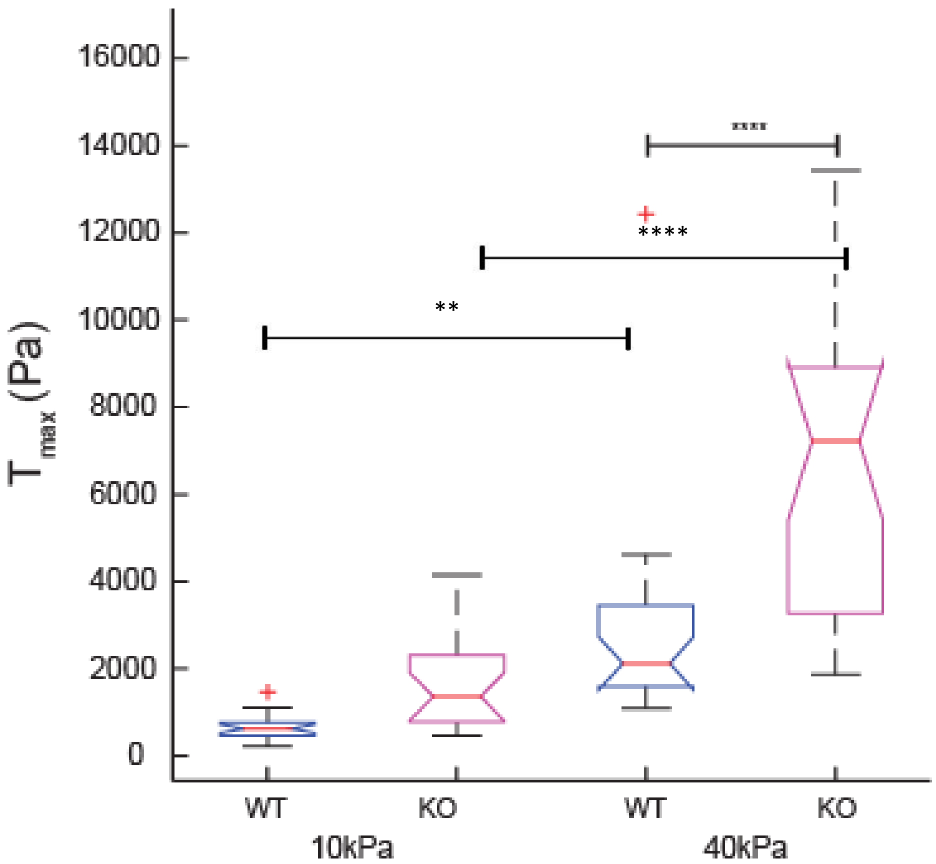
Unconstrained Traction force generated by FTTC code

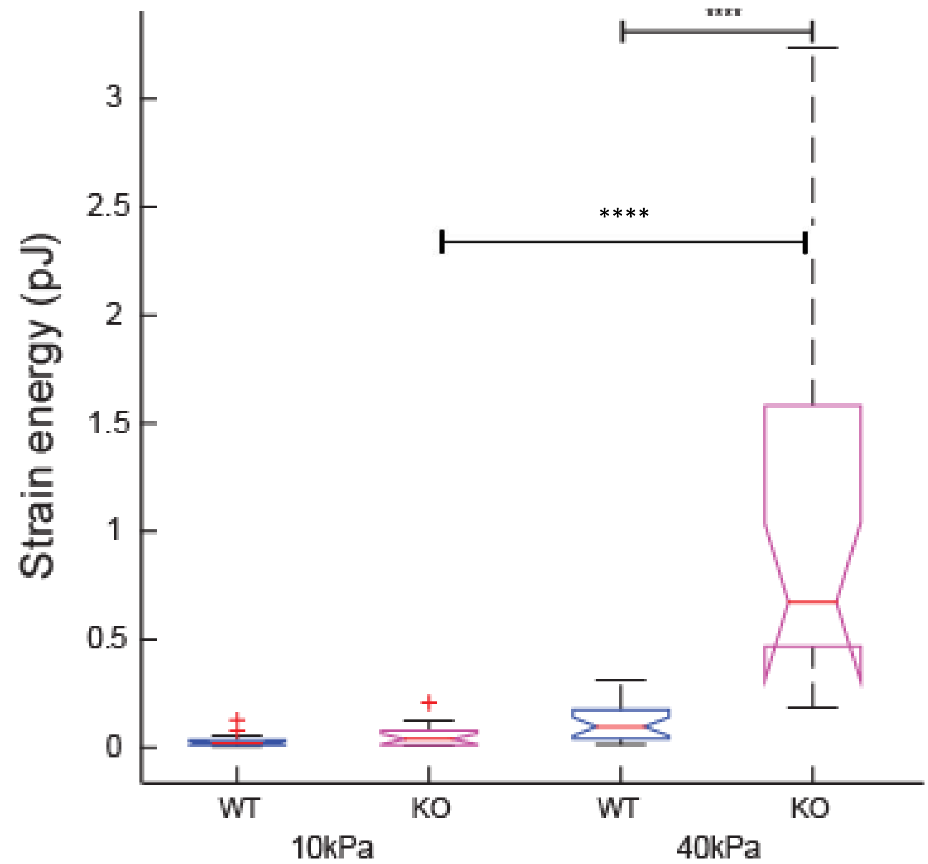
Traction – statistical analysis

- ANOVA with Bonferroni multicompare test
- Confidence interval > 95 %
- Box Plots

n > 15

Traction and Strain energy





Significant difference in

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Significant difference in

- ✓ WT 10 kPA & WT 40 kPa
- ✓ KO 10 kPa & KO 40 kPa
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Conclusion

- ✓ **Significant difference in morphology** of WT and KO cells
  KO cells have more cell area and nuclear area, but less nuclear ellipticity

- ✓ **Tractions and strain energy of KO cells is significantly higher** than WT cells

- ✓ Traction results are correlated to cell morphology results

- ✓ This results are **contradictory to what we expected-** due to loss of FA sites in low Cav expression

- ✓ **cell line specific** function of caveolin

- ✓ Loss of Caveolin protein may cause **change in nature and distribution of FA** sites

- ✓ Loss of Caveolin expression **may upregulate some other unknown compensatory mechanisms** which is causing increase of tractions in KO

Future directions….

- Validate our results by expressing Cav1 in KO cells & observe rescue phenotype
- To see distribution and area of FA sites in KO cells by antibody staining of FA protein such as integrin
- Inhibitor study on actin and myosin of KO cells to check change in cytoskeletal contractility

Acknowledgement

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THANK
YOU
JUSPAXAR
OBRIGADO
MATONDO
SALAMAT
KIITOS
KIA ORA
MULTUMESC
MAAKE
MOCHCHAKKERAM
TERMA KASIH
RAIBH MAITH AGAT
CHOKRANE
SALAMAT
GRAZIE
MULTUMESC
MERCI
CAM ON BAN
MERCI
RAIBH MAITH AGAT
MERCI
MOCHCHAKKERAM
OBRIGADO
MOCHCHAKKERAM
GRAZIE
ASANTE
CHOKRANE
UA TSAUG RAU KOJ
MATUR NUWUN
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KIITOS
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DANK JE
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