STUDY SUB-NANOMETER MEMBRANE FLUCTUATIONS IN SINGLE CELLS USING A PLASMONIC IMAGING MICROSCOPE

A THESIS BY
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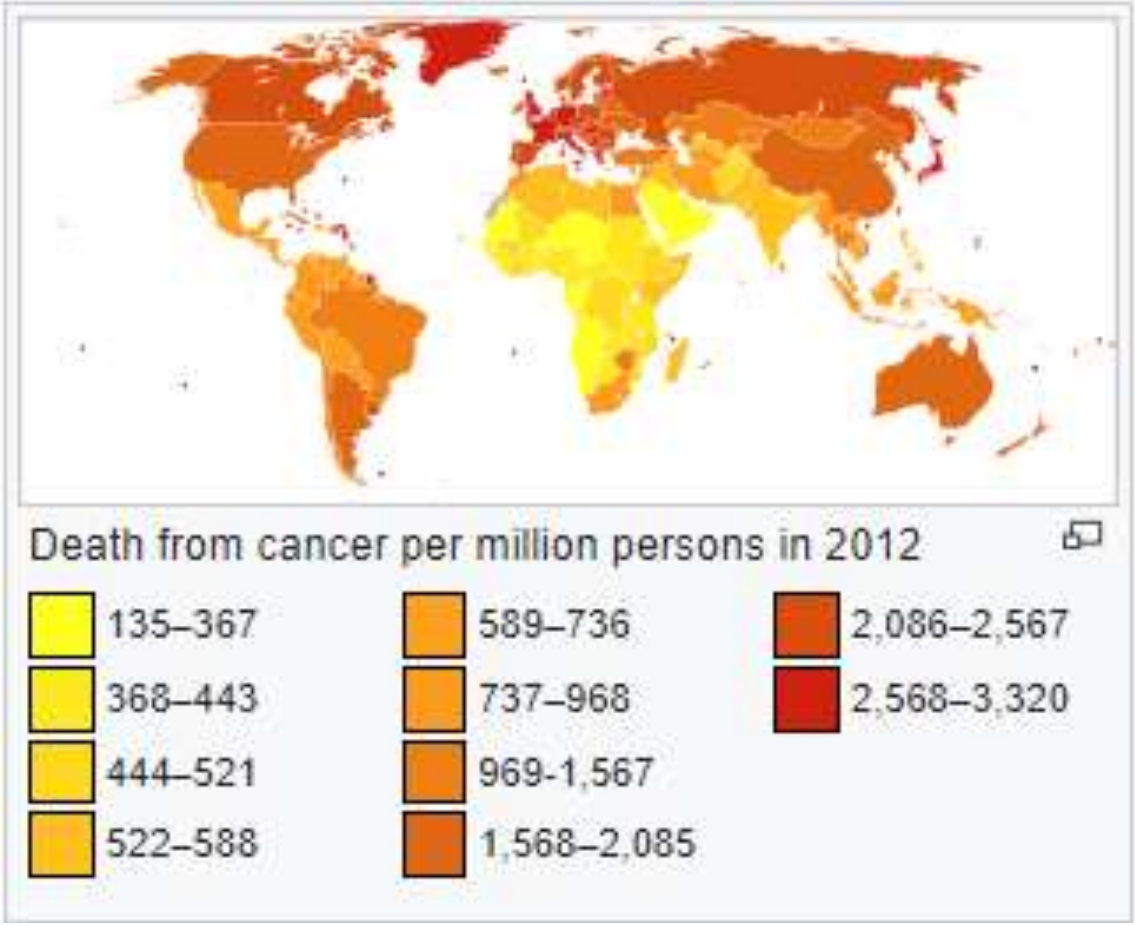
Outline:

1. Background
2. Surface Plasmon Resonance Imaging
3. Sub-Nanometer Membrane Fluctuations
4. Cell Heterogeneity
5. Cell Metastasis
6. Conclusion
7. Future Scope

Background

World Health Organization Report



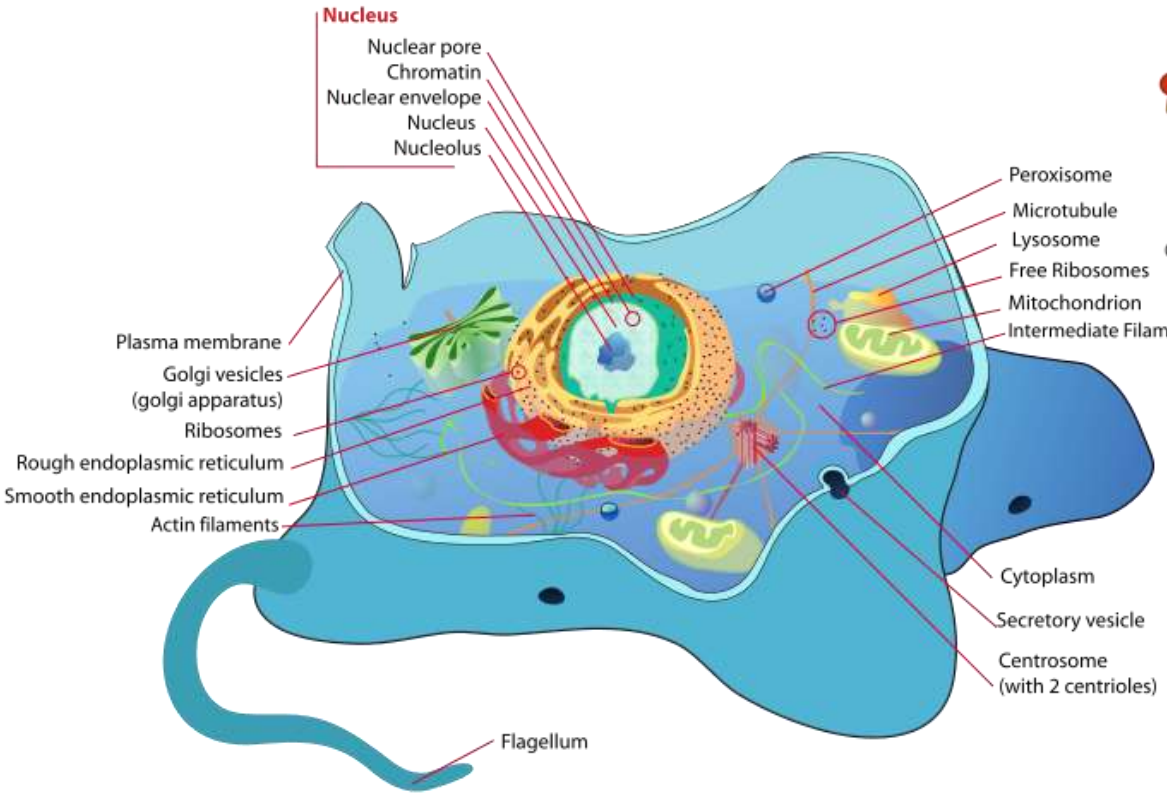
According to a survey by American Cancer Society in 2017,

- 15.5 million Americans with a history of cancer were alive on January 1 , 2016.
- Around 1.6 million people were expected to be diagnosed with cancer in 2017 (excluding the ones with non-invasive cancer).
- Around 0.5 million were expected to die of cancer in 2017 (1650 people per day).

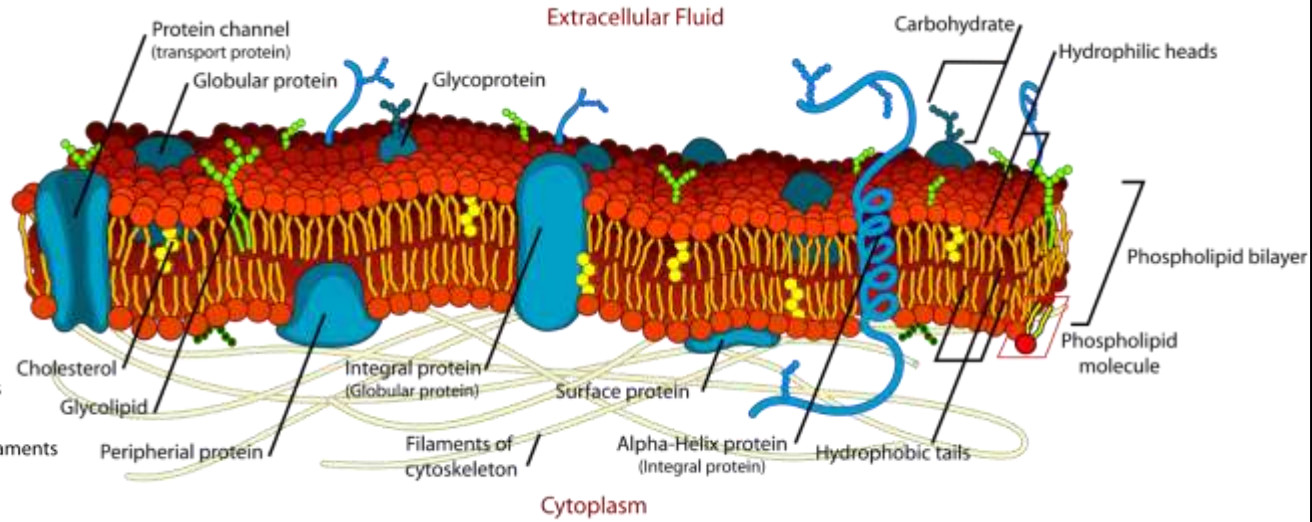
Do we know the reason for cancer? | If not, can we study cancer cells? | Can we find a remedy?

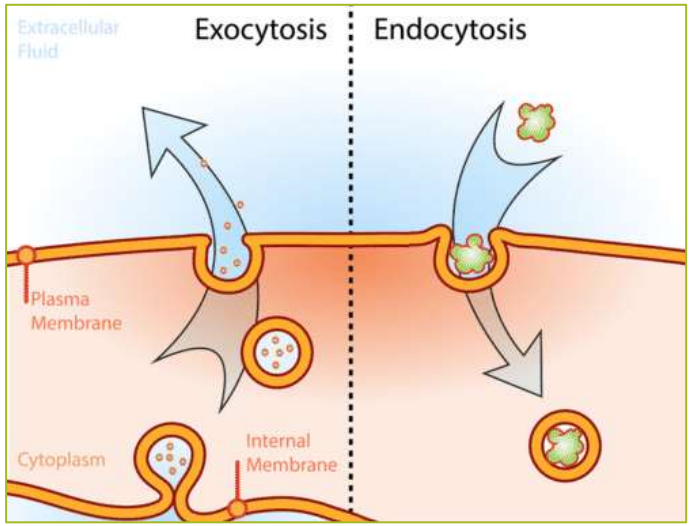
Introduction to Membrane Fluctuations:

Structure of a Biological Cell



Cause of Cell Membrane Fluctuations





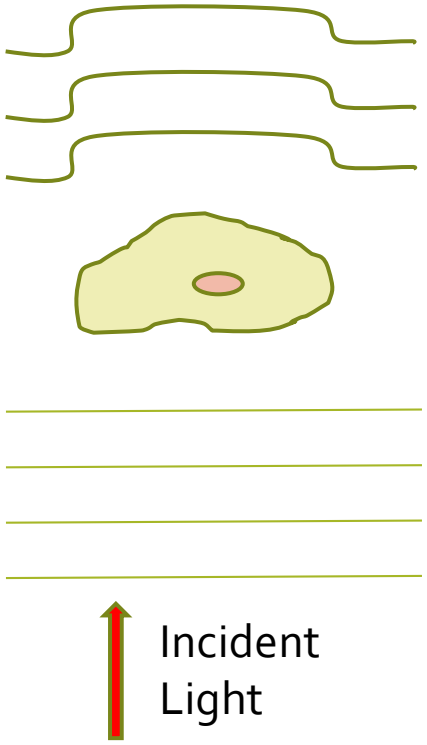
- ✓ Live cells undergo continuous <u>active processes</u>.
- ✓ **Ex.** :- Metabolism, Metastasis, Mitosis, etc.

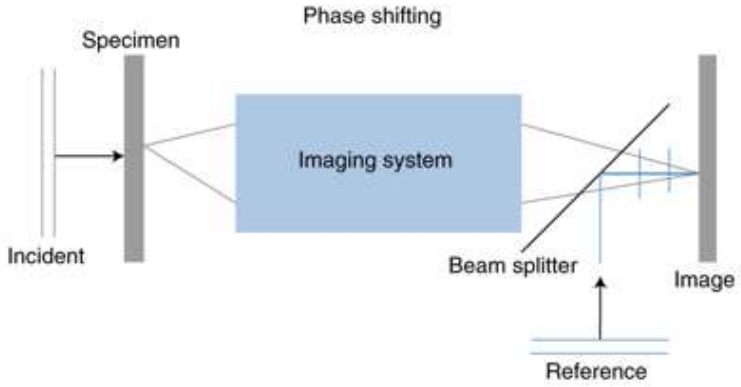
Active fluctuations:

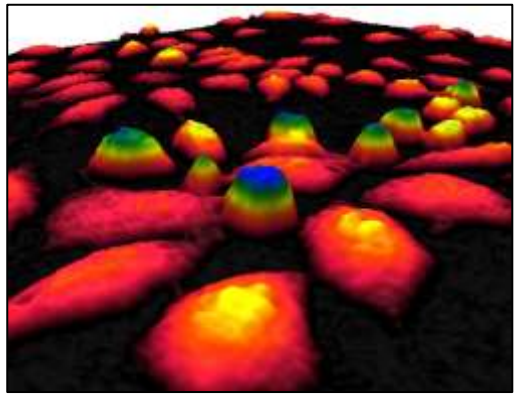
- ✓ Cross-membrane ion-transport.
- ✓ Cell structure
- ✓ Endocytosis and Exocytosis

Techniques to Image Membrane Fluctuations:

Quantitative Phase Imaging

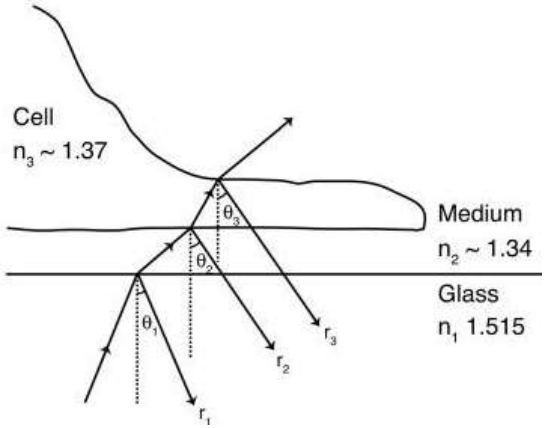


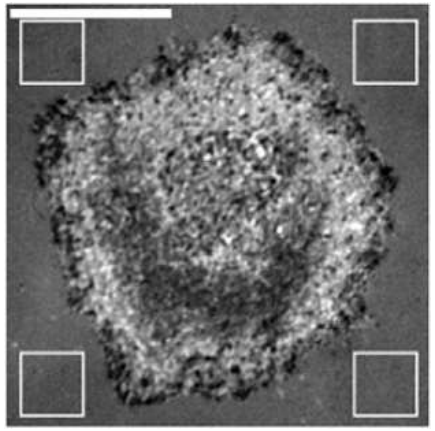


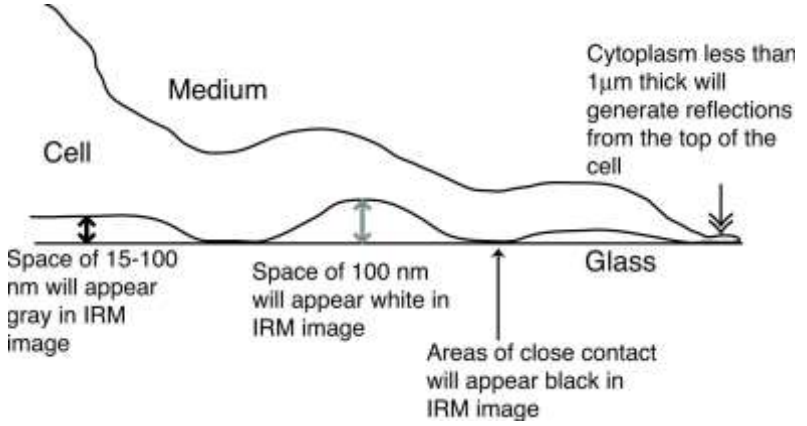


QPI Image

Interference Reflectance Microscopy





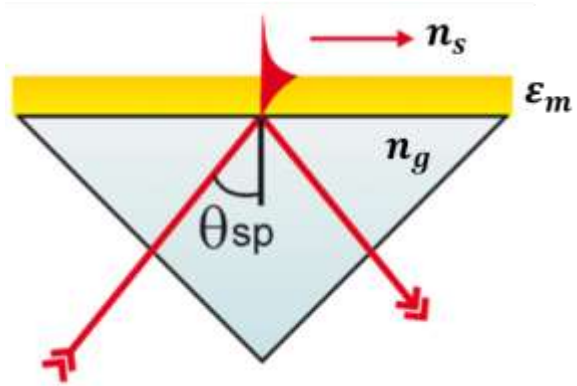


Our Approach: Surface Plasmon Resonance Imaging

Sensitive only to bottom membrane	• Image only the bottom cell membrane. • High sensitivity in z-direction, less distortion.
High Sensitivity : Sub-nanometer Membrane Fluctuations	• Study metabolism. • Study metastasis.
Single Cells	• Study fundamental properties. • Study cell heterogeneity.

Principle of Surface Plasmon Resonance (SPR) Imaging

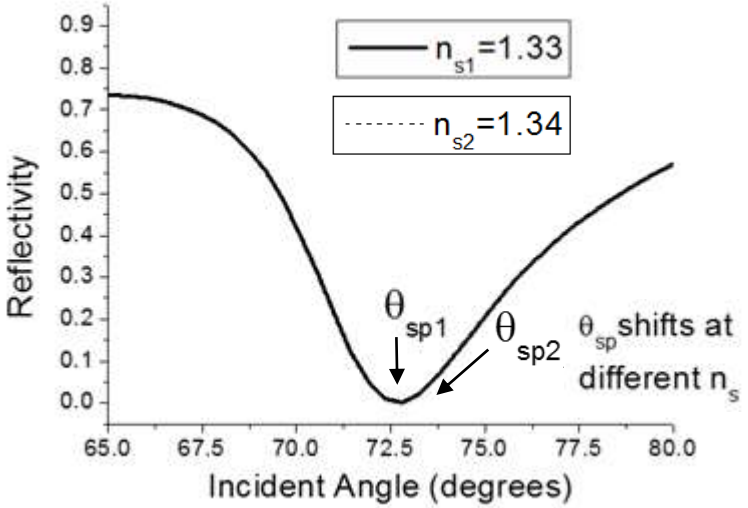
✓ High Sensitivity



SPR Interface

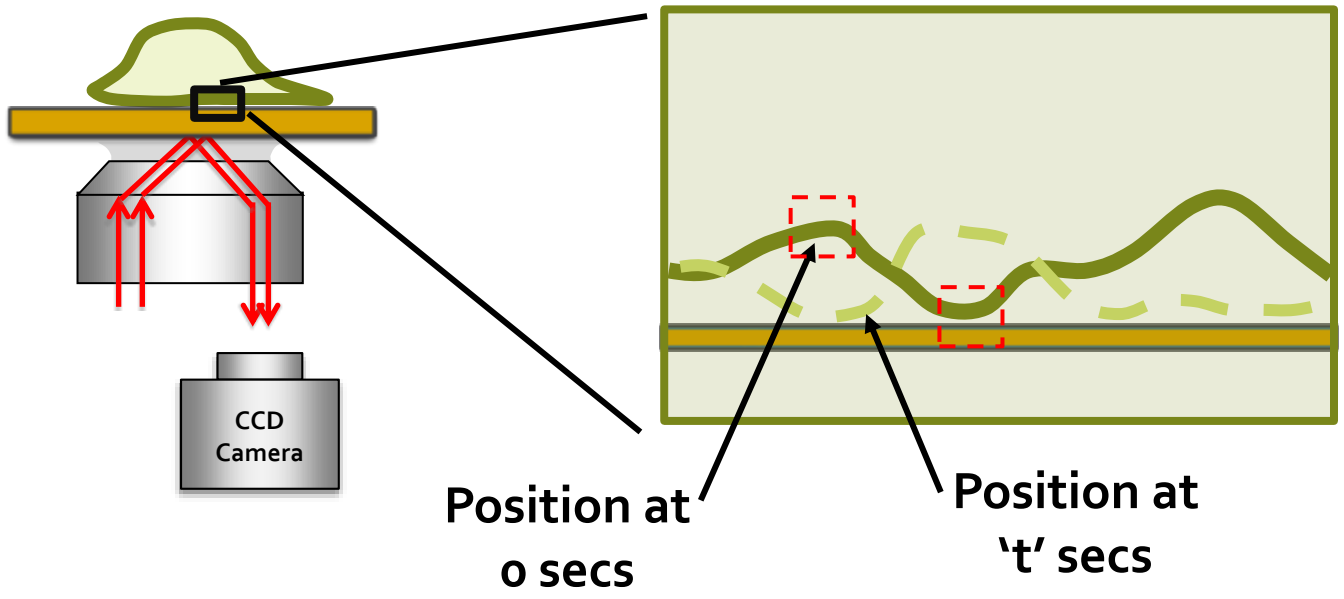
$$\sin\theta_{sp} = \frac{\sqrt{\frac{\varepsilon_m n_s^2}{\varepsilon_m + n_s^2}}}{n_g}$$

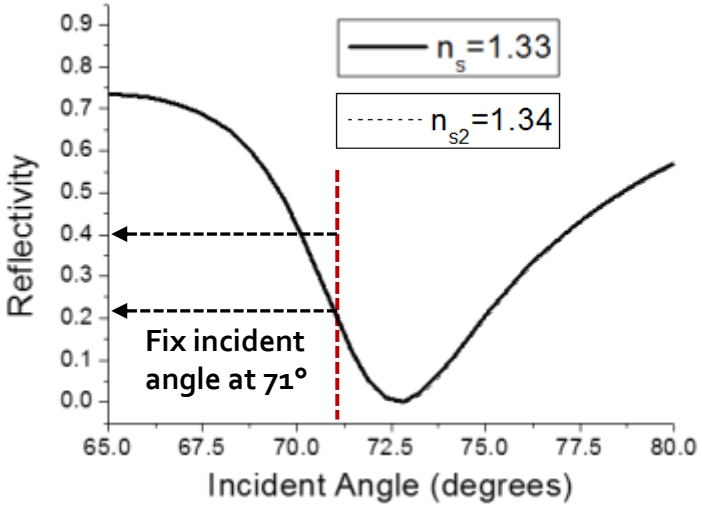
SPR Angle



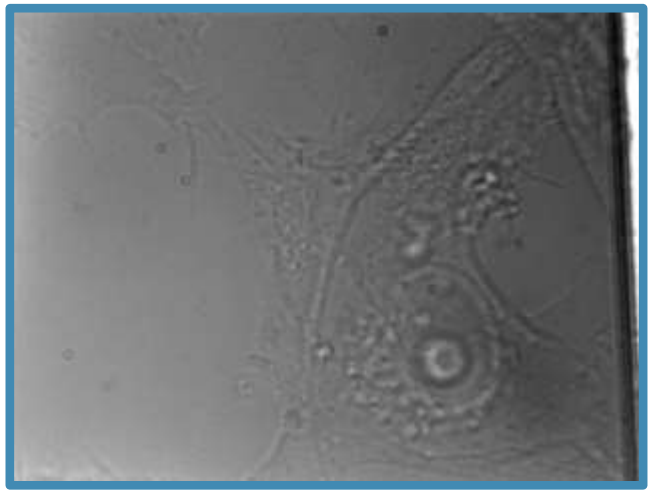
SPR Response

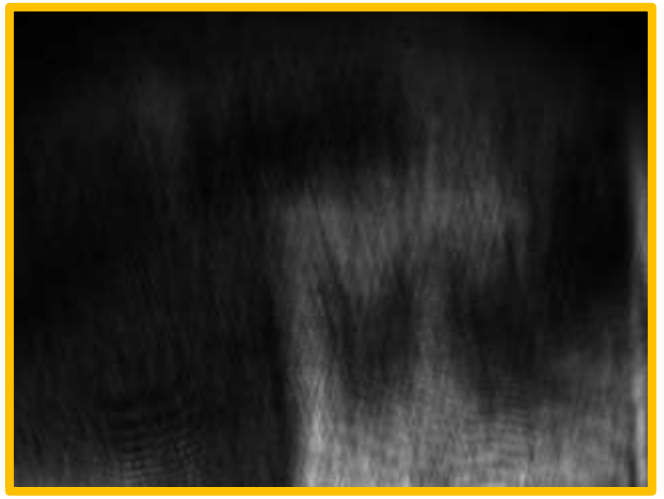
Live Cell Imaging Using SPR





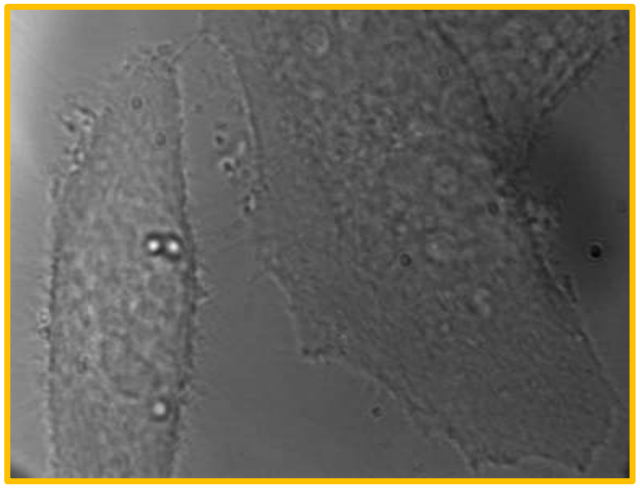
Transmitted Image

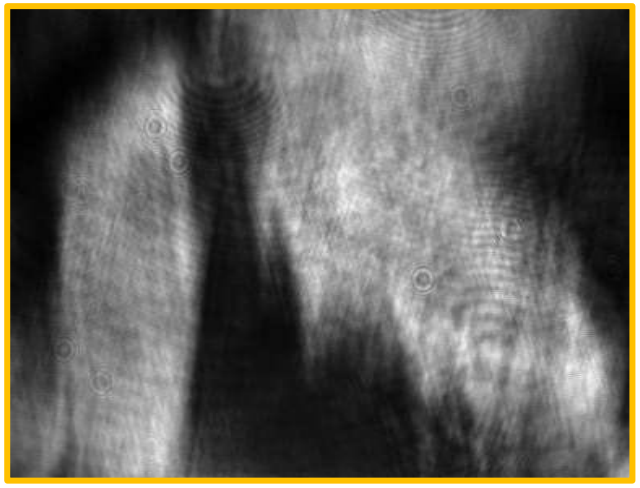


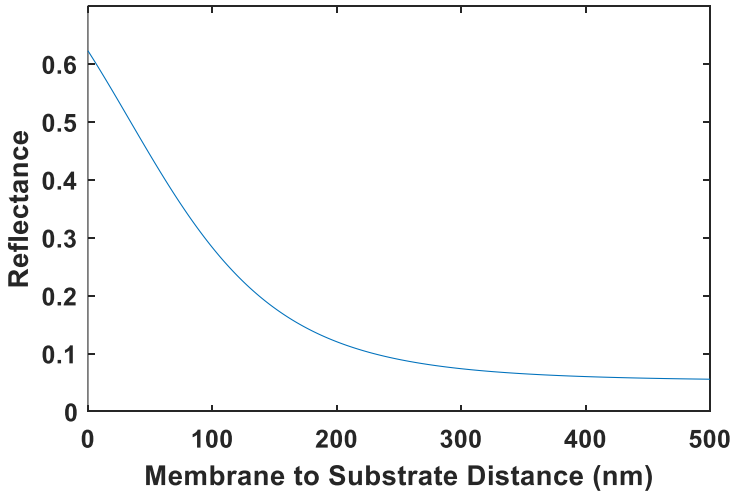


SPR Image

Mapping Reflected Light Intensity to Distance from Substrate



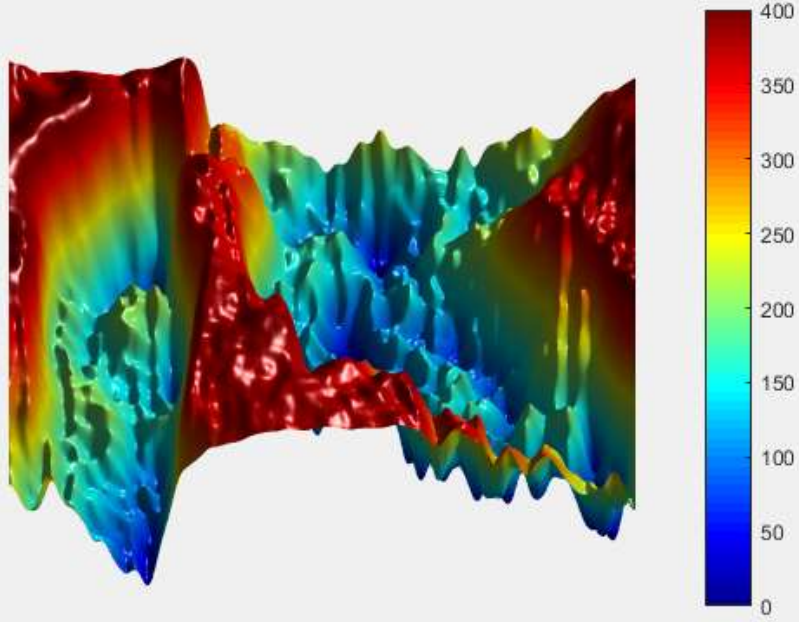




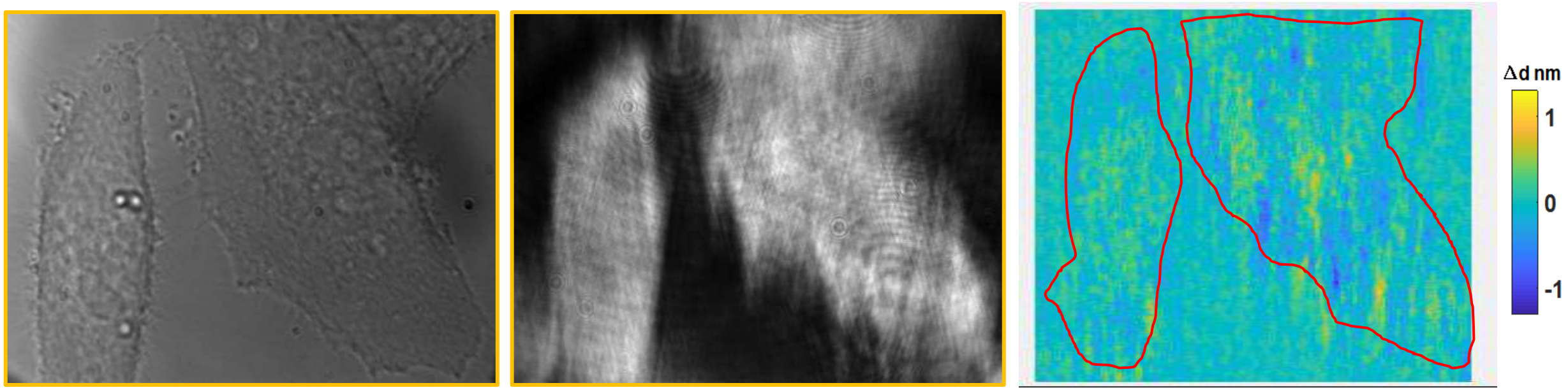
Calibration Curve

Simulation Model

Layer 5	Cytoplasm
Layer 4	Cell Membrane
Layer 3	Culture Medium
Layer 2	Au
Layer 1	BK7

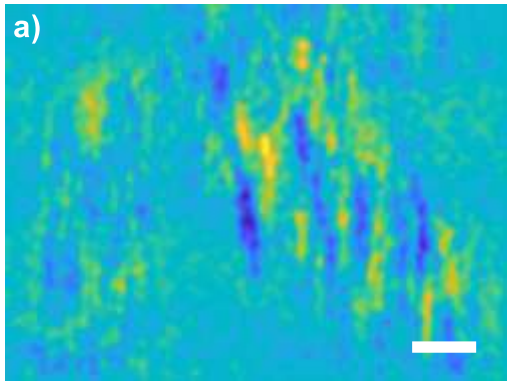


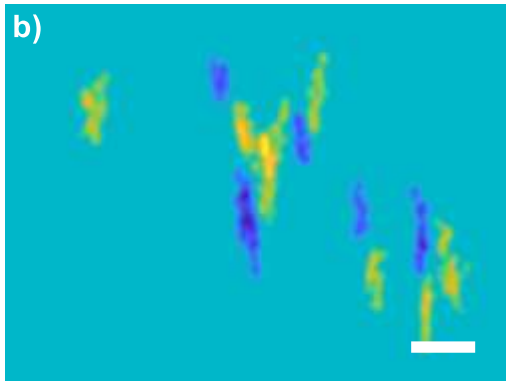
Imaging the Sub-nanometer Membrane Fluctuations

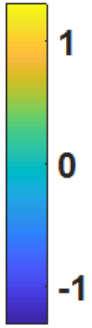


Analyse Sub-Nanometer Membrane Fluctuations

t = 5 sec







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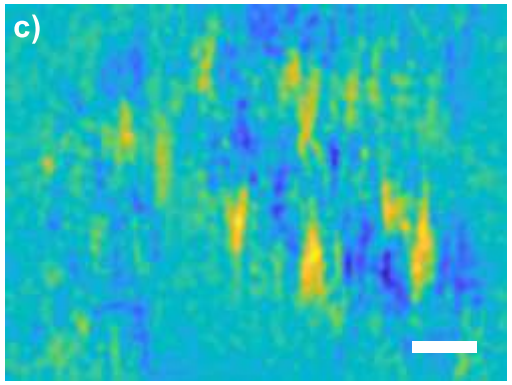

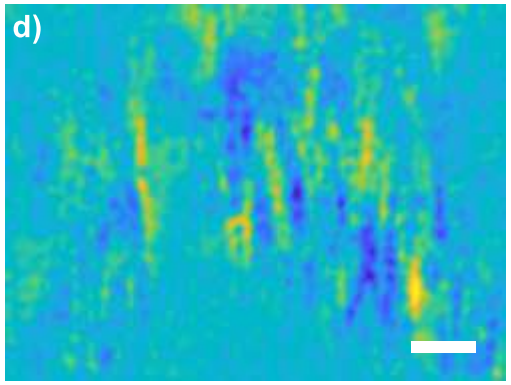

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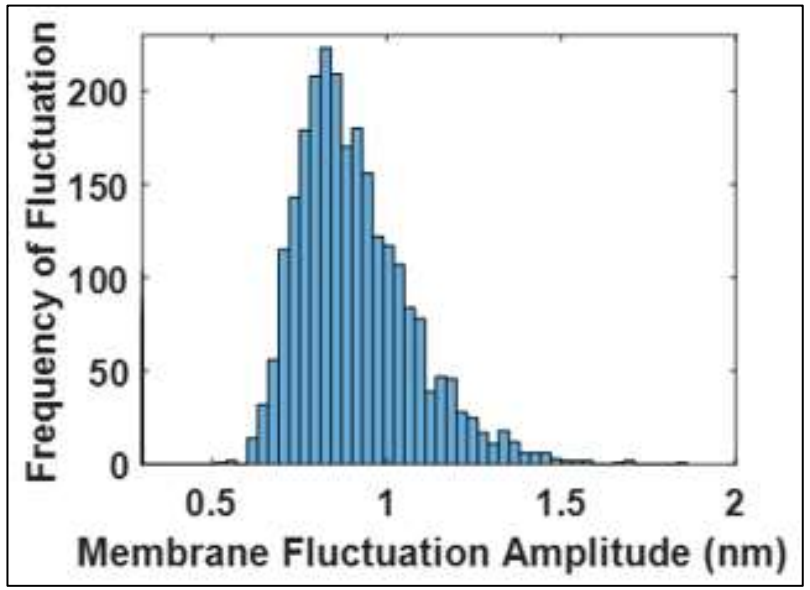


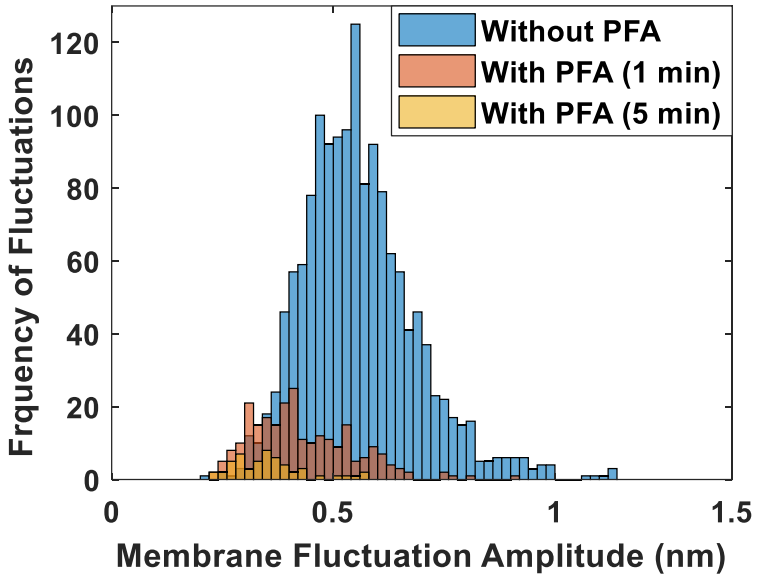
Scale Bar = 10 µm

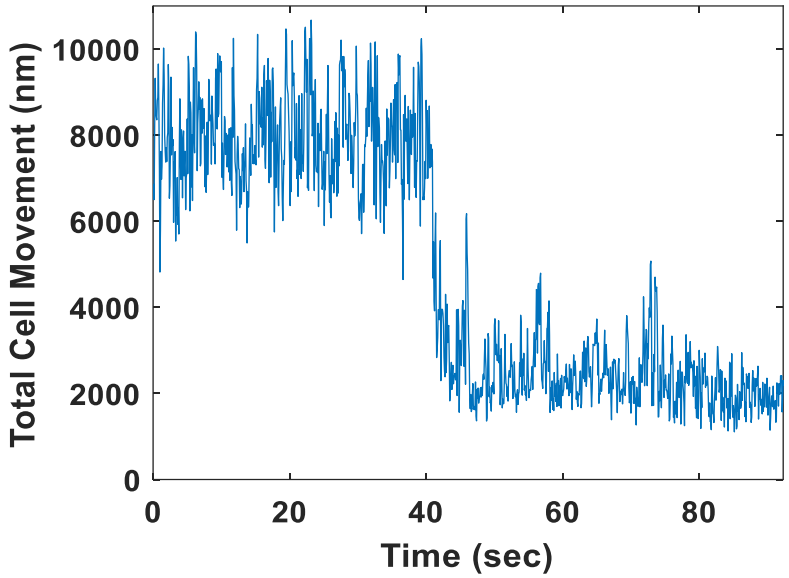
- ✓ Image small yet swift movement.
- ✓ Distribution range for fluctuations.
- ✓ These movements may contribute to respiration, metabolism, active fluctuations and thermal fluctuations.

Confirm Active Membrane Movement By Cell Fixation

- Add Paraformaldehyde (4% in PBS)

Add 100 µl PFA in 200 µl Culture Medium (50% Conc.)

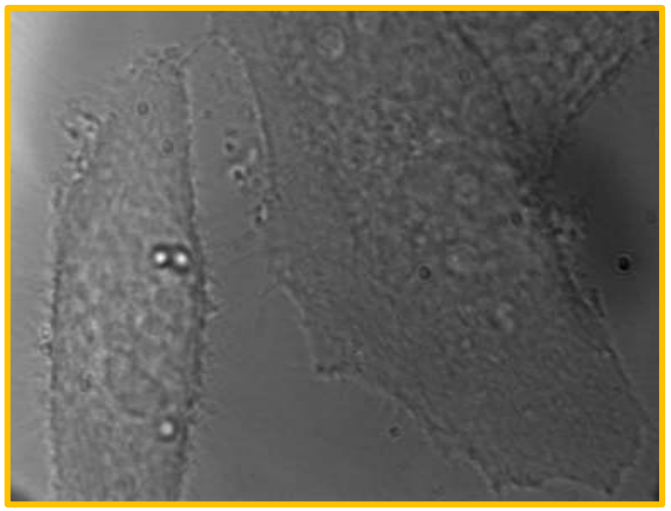


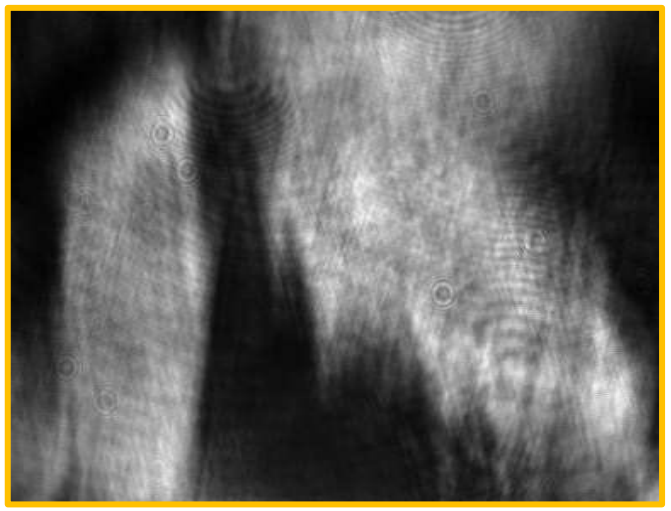


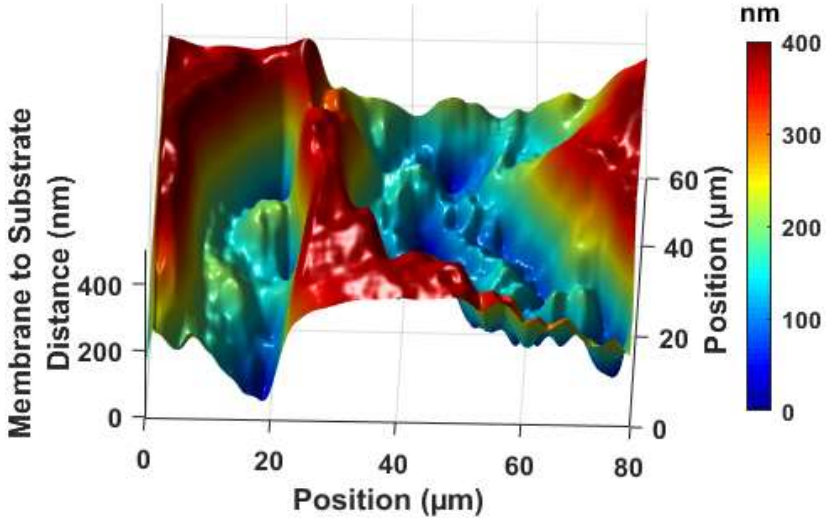
Record Entire Fixation Process

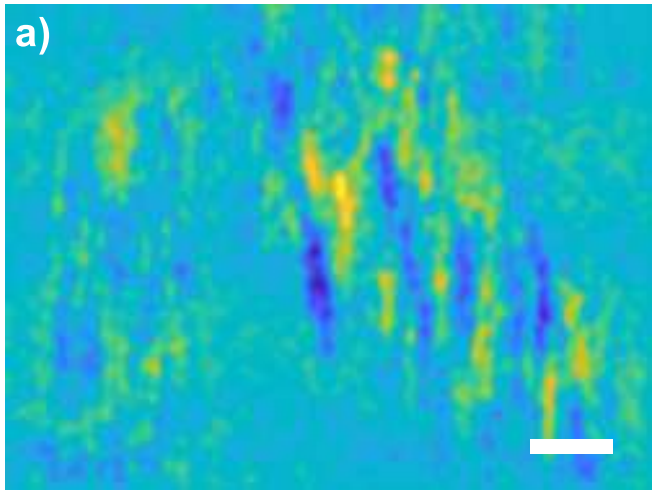
- Membrane Fluctuations: Brownian or Active?
- Highly concentrated dose kills the cells.
- Therefore, expect no membrane fluctuations.
- This confirms that we image active movement.

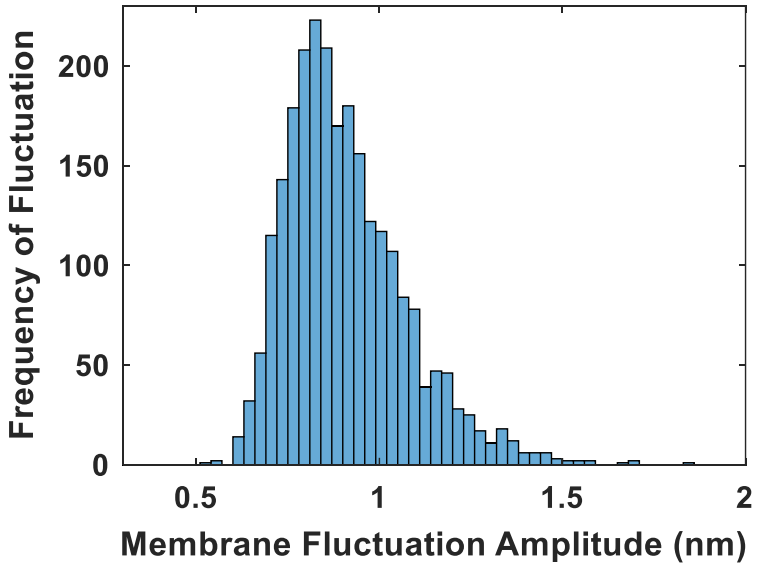
Quick Review

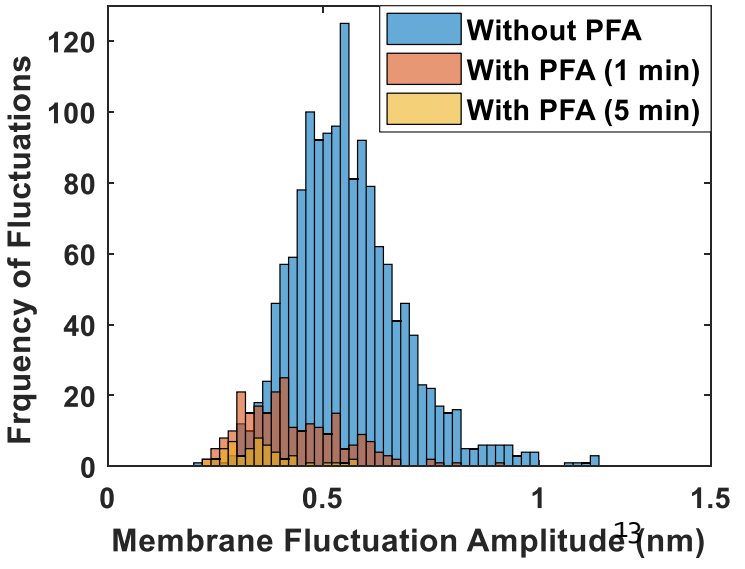






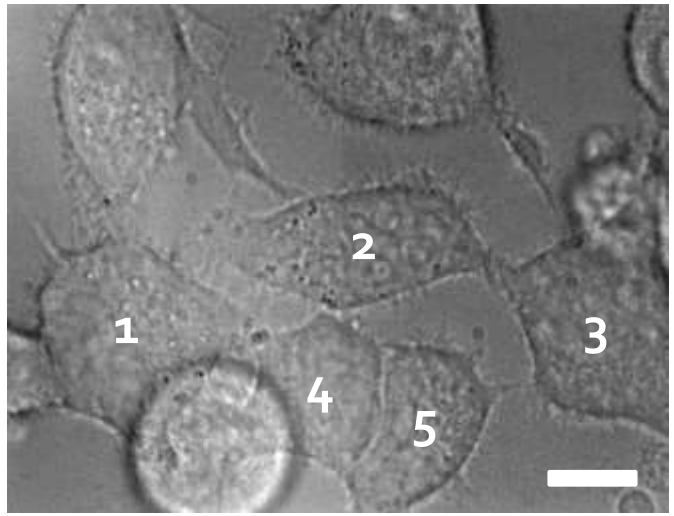






Cell Heterogeneity

a)



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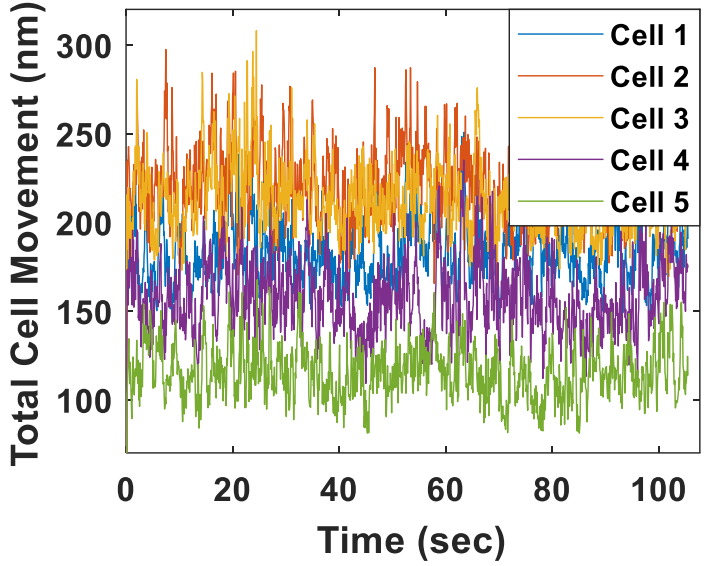
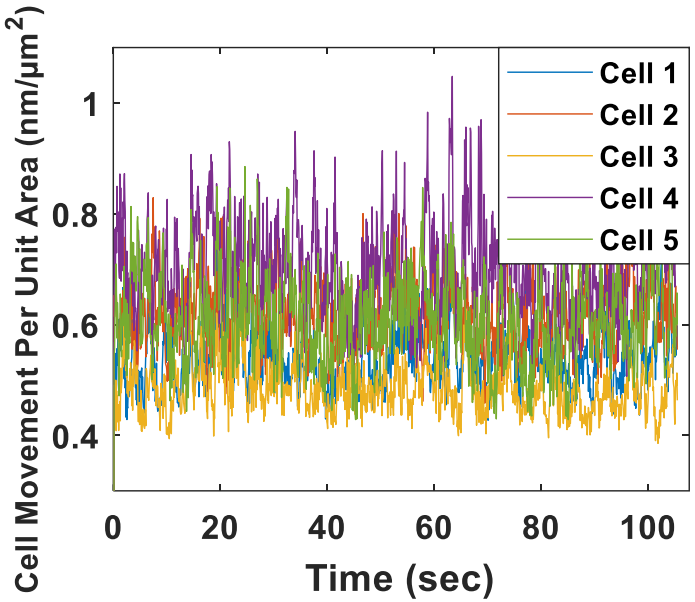


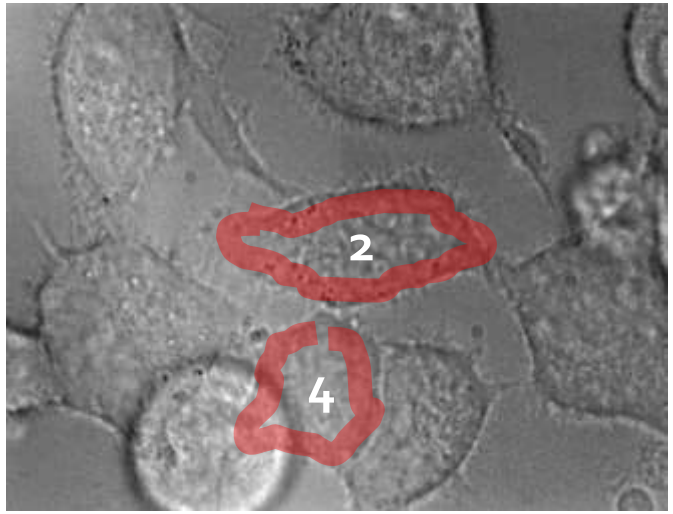
c)

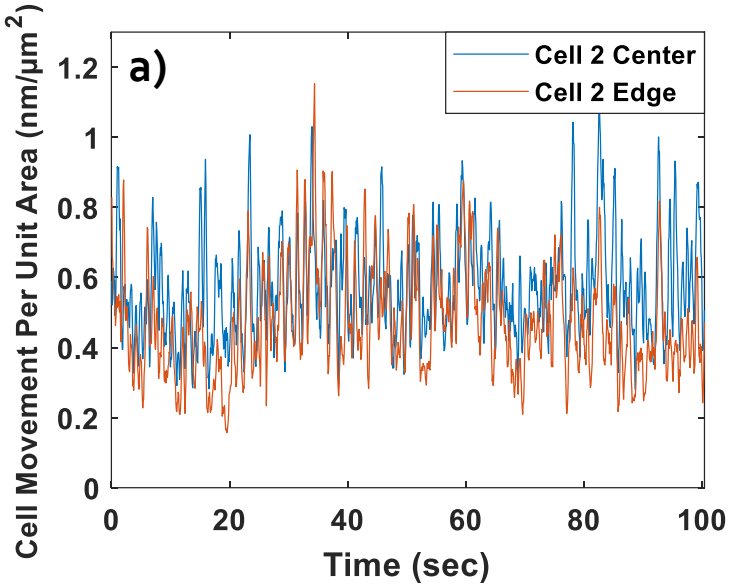


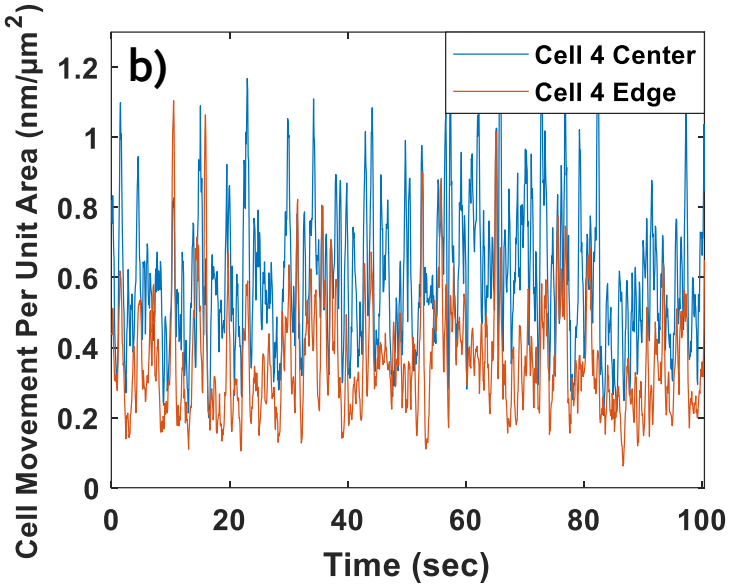
- ✓ Characteristics of a population may not reflect characteristics of an individual cell.
- ✓ Different cells are at different stages of growth at a given time point.
- ✓ **Factors Involved:** Environmental Conditions, Genetic Variations, interaction with the surrounding.

Intra-Cell Correlation and Differences

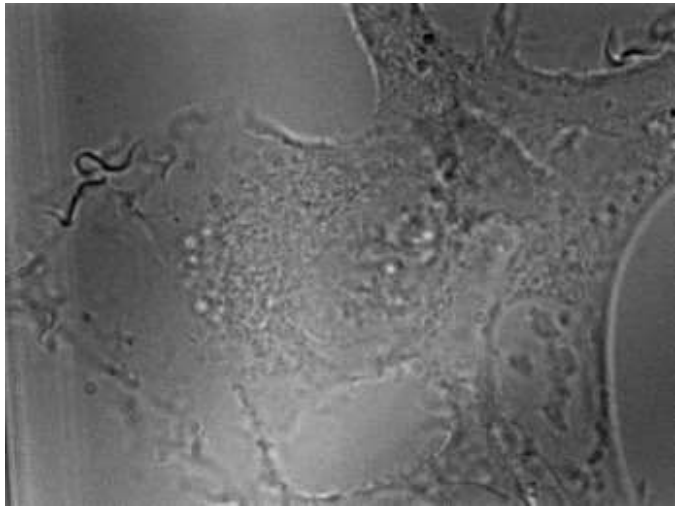
HeLa Cells







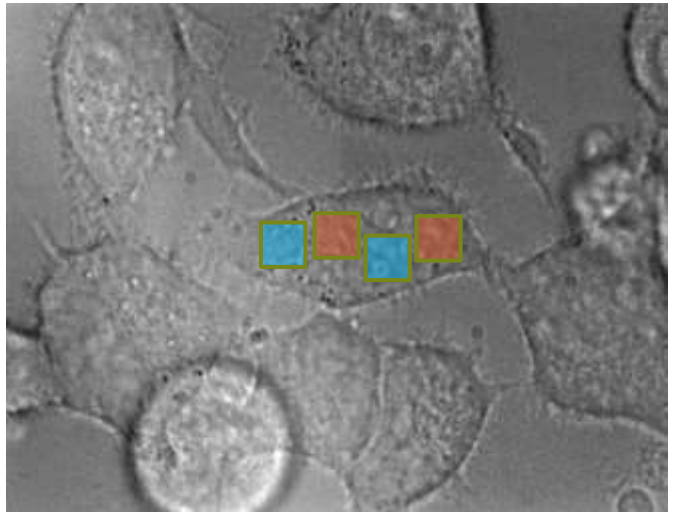
HT-1080 Cells



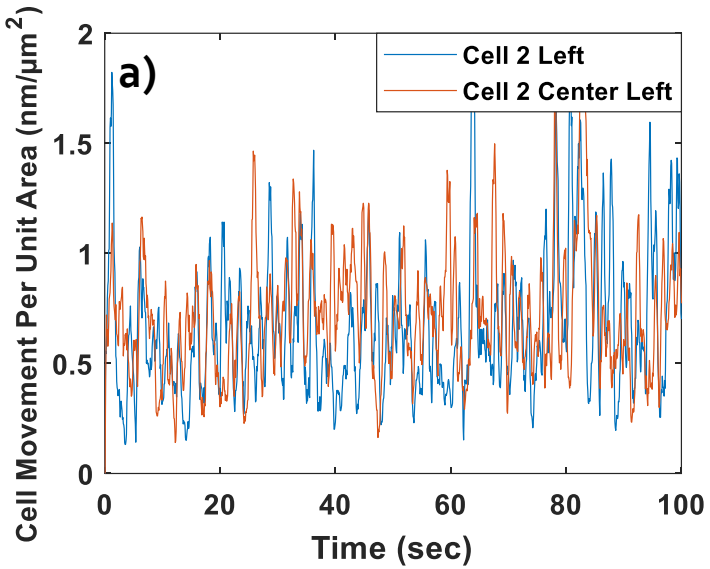
- ✓ Center is more active than edges.
- ✓ Observe energy expenditure.

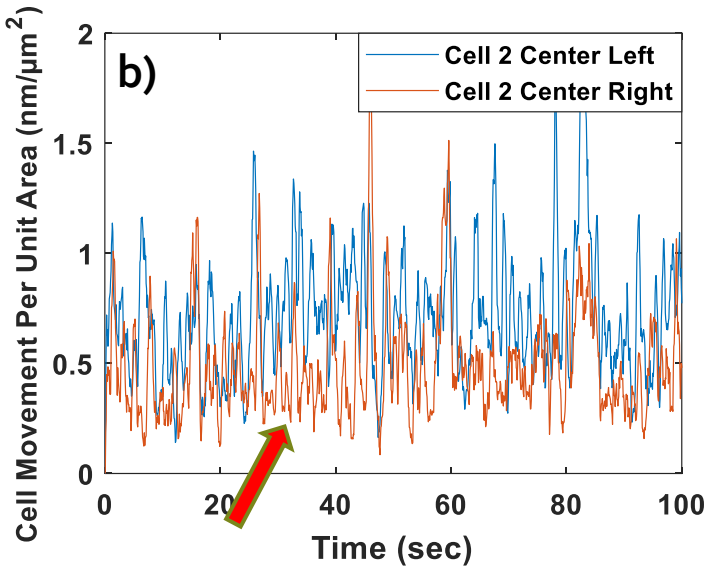
Intra-Cell Transient Heterogeneity

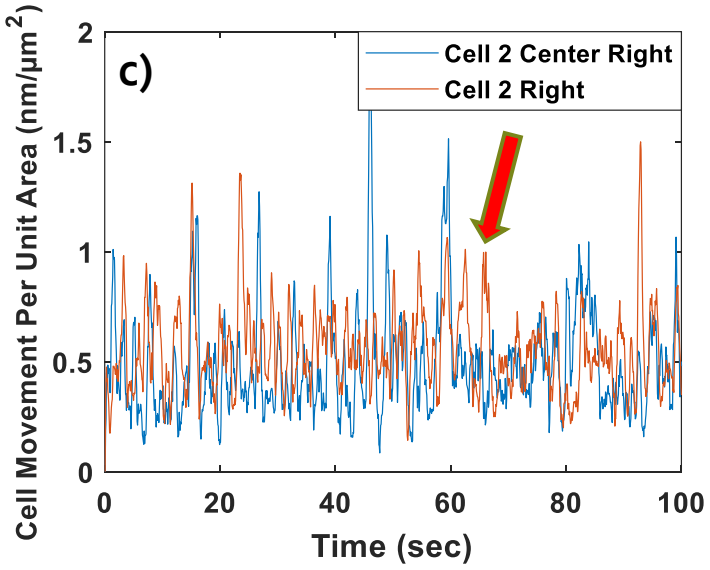
HeLa Cells

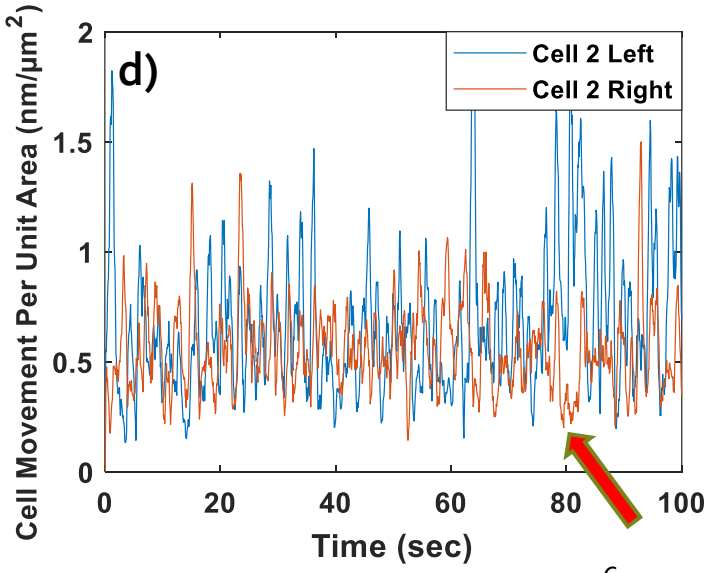


- Analyse sub-regions (5 x 5 µm).
- Transient heterogeneity.
- Related to active processes.





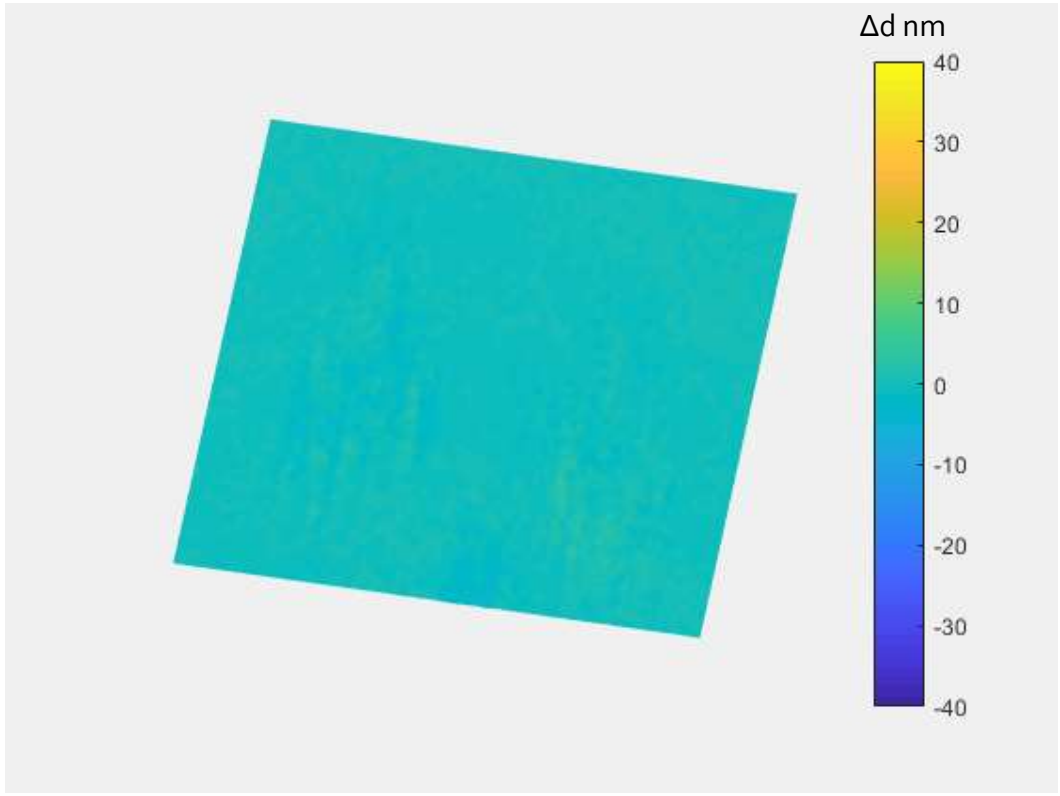


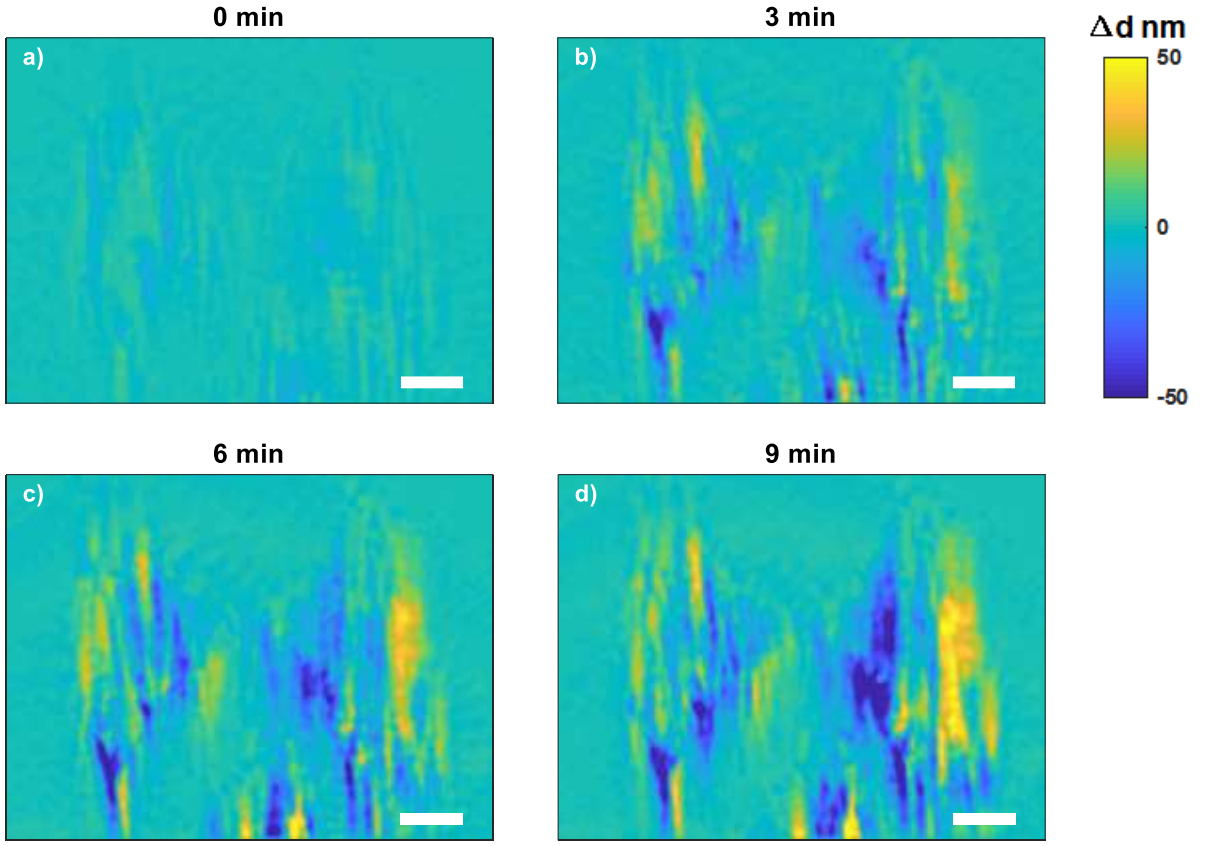


Long Duration Cell Membrane Movement

(Scale Bar = 10 μm)

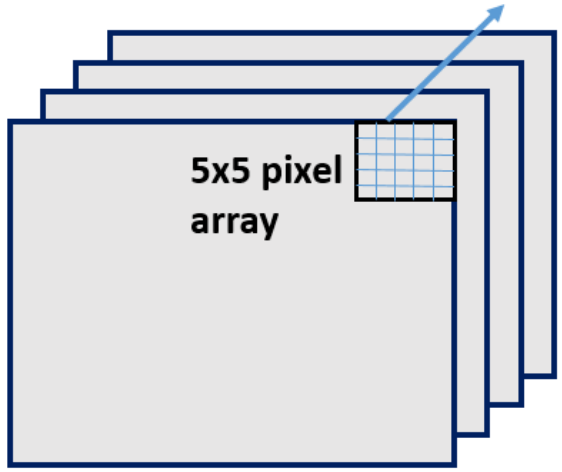
3D Video Displaying Membrane Movement

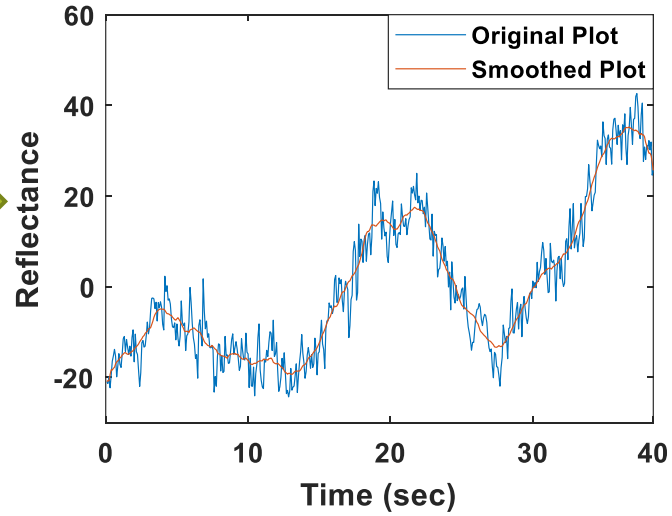


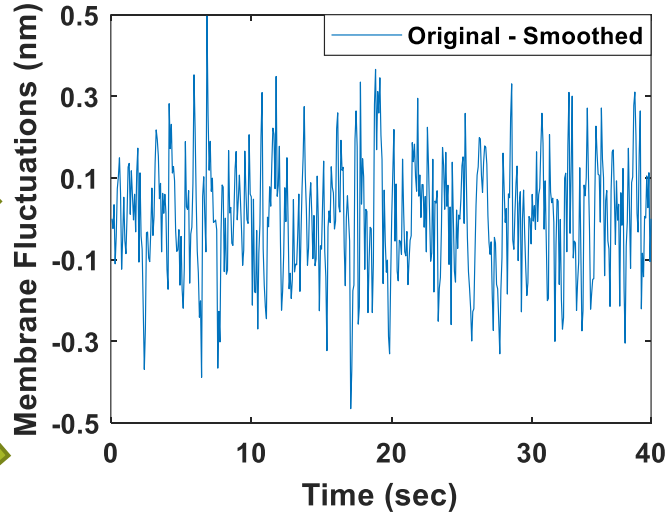


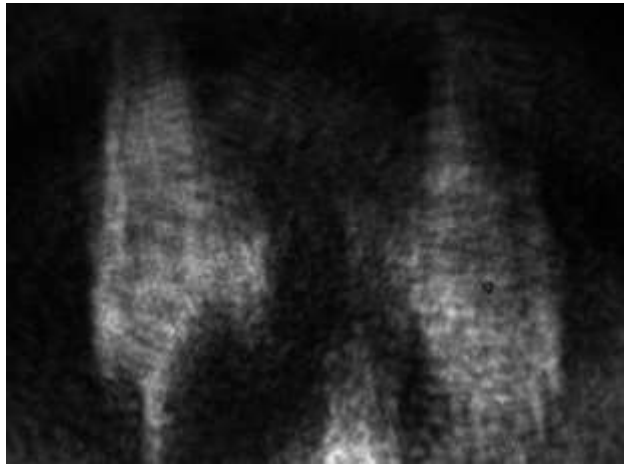
- ✓ We see a collective movement (10's of nanometers) as we record for longer time.
- ✓ This can be related to physiological processes like metastasis.
- ✓ Do they have a correlation with the sub-nanometer membrane fluctuations?

Relation Between Membrane Movement and Sub-nanometer Fluctuations

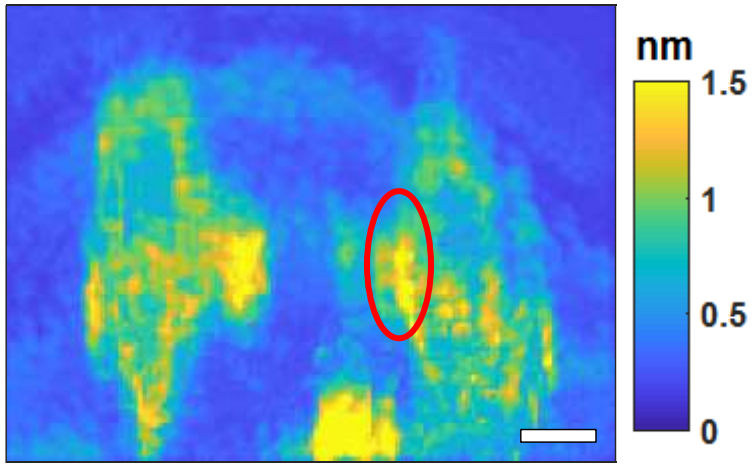




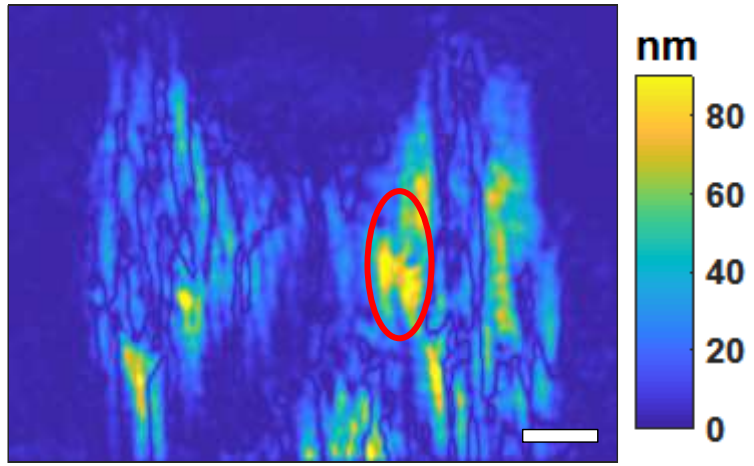




SPR Image



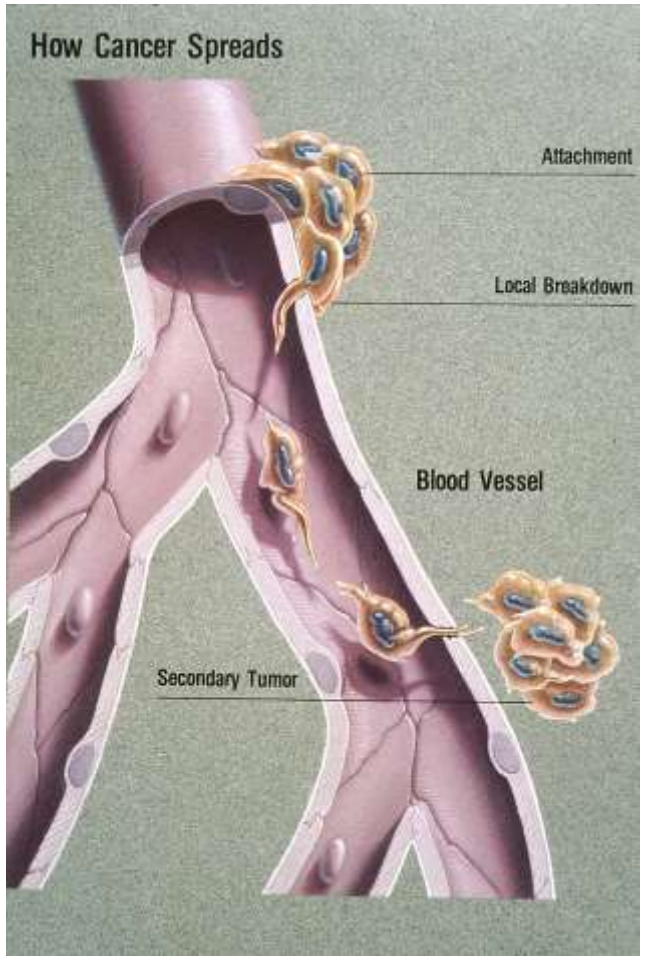
Sub-nanometer Fluctuation Map



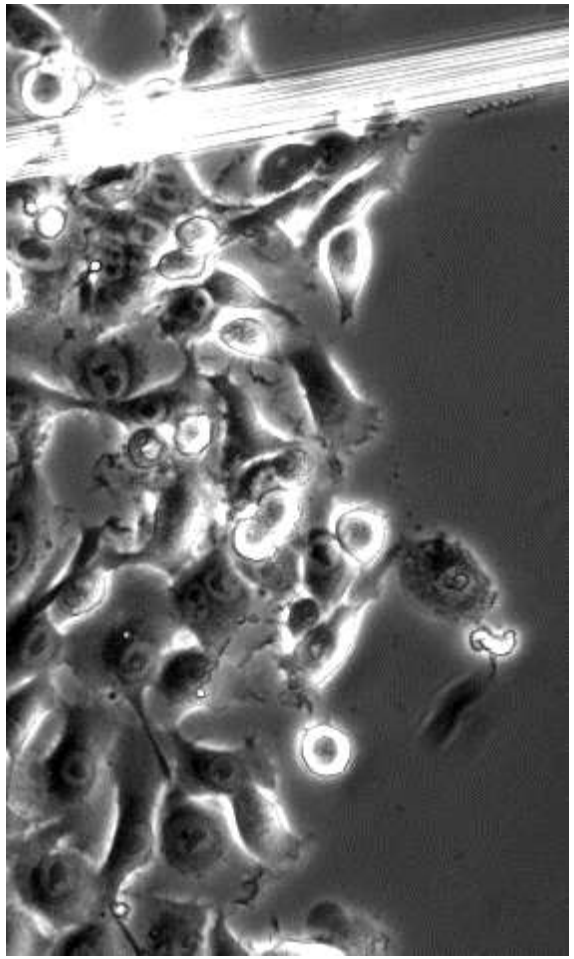
Membrane Movement Map

- Both movements are correlated to some extent.

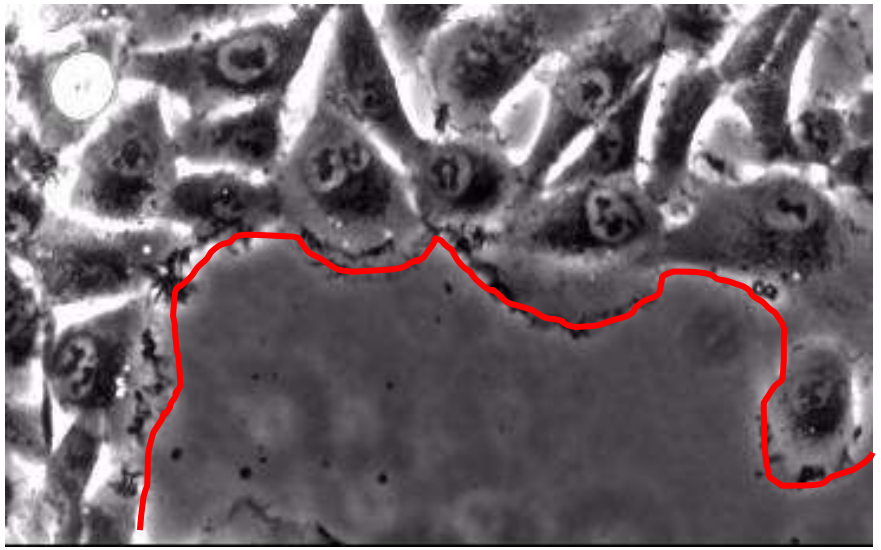
Cell Metastasis



HT-1080



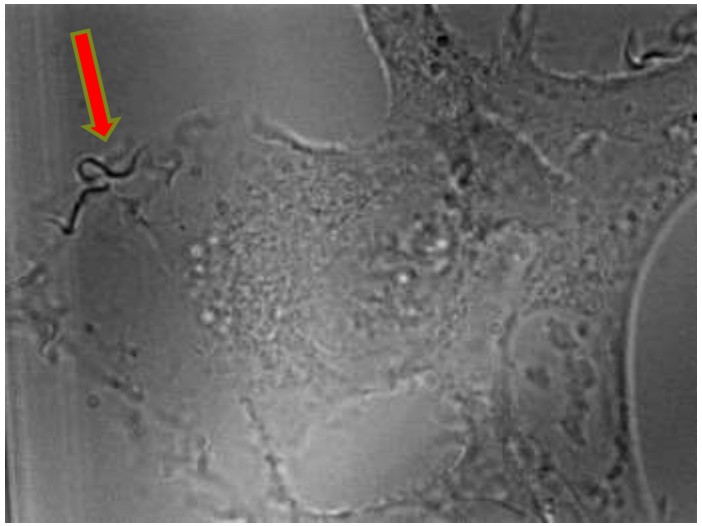
Wound Healing Assay



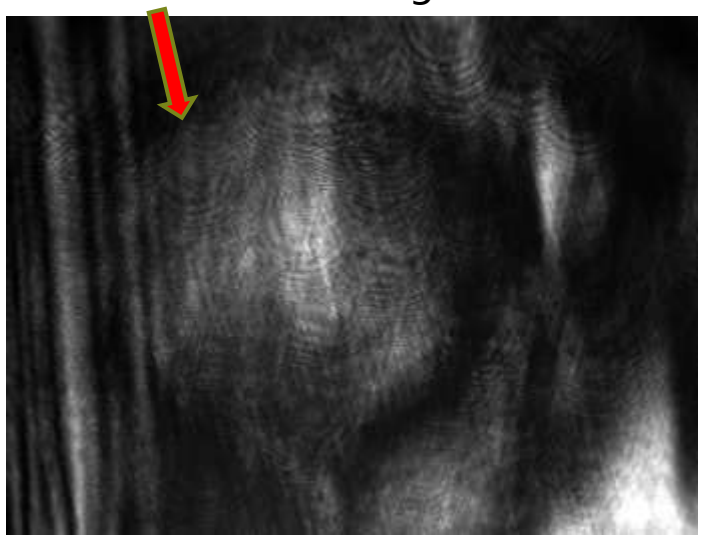
- ✓ HT-1080: Human Fibrosarcoma Cell Line.
- ✓ Highly active cell membrane fluctuations.

Membrane Movement in Metastatic Cells

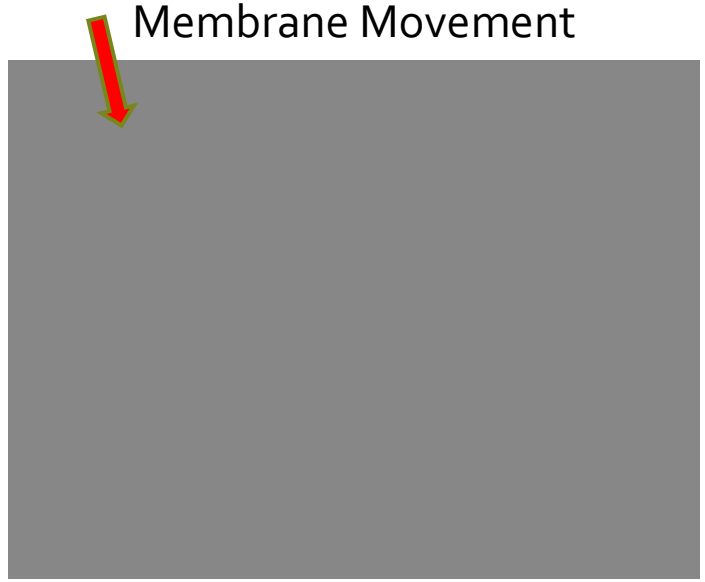
Bright-Field Image

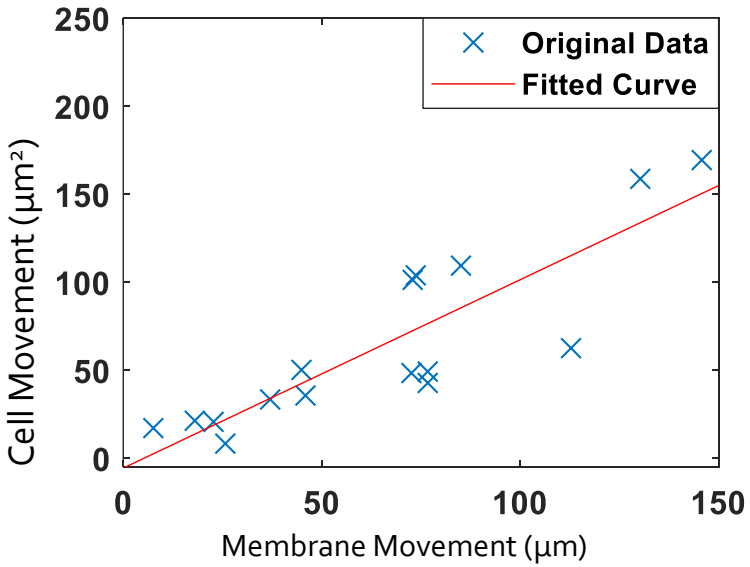


SPR Image



Membrane Movement





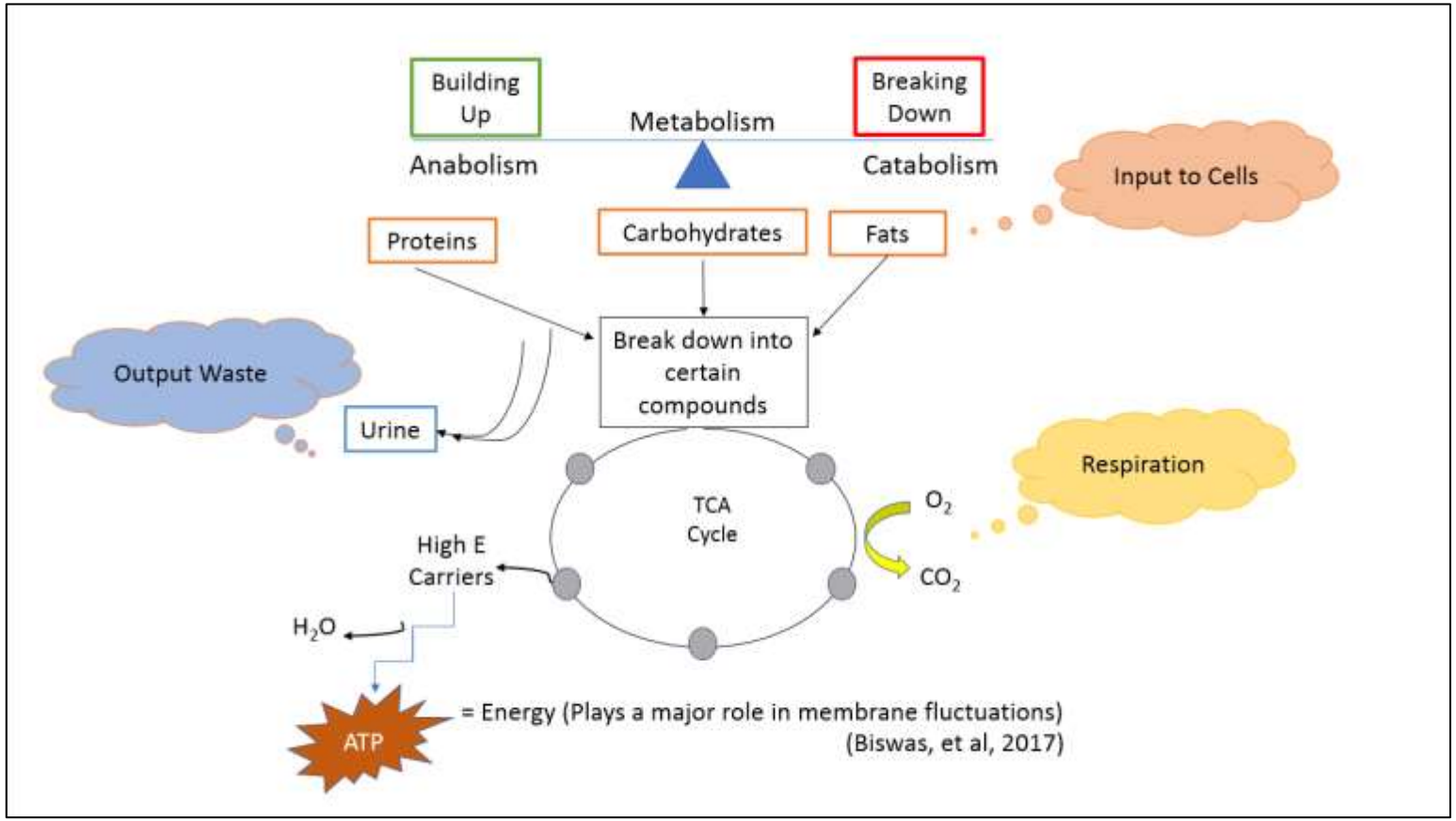
Displaced Area α Membrane Movement

Conclusion:

- ✓ Live cell imaging of cell bottom membrane with high resolution in z-direction and less noise.
- ✓ Imaging the cell membrane fluctuations important to study metabolism.
- ✓ We observe heterogeneity in cells which corresponds to multiple factors.
- ✓ Enable to analyse metabolic response of a cell during anti-cancer drug treatments.
- ✓ Cell metastasis can be evaluated based on membrane fluctuations to some extent.

Future Plan

1) Using membrane fluctuations as a characteristics to study metabolic activities at single cell level.



2) Analyse response of cells to anti-cancer drugs targeting active processes.

Thank You…!!!