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

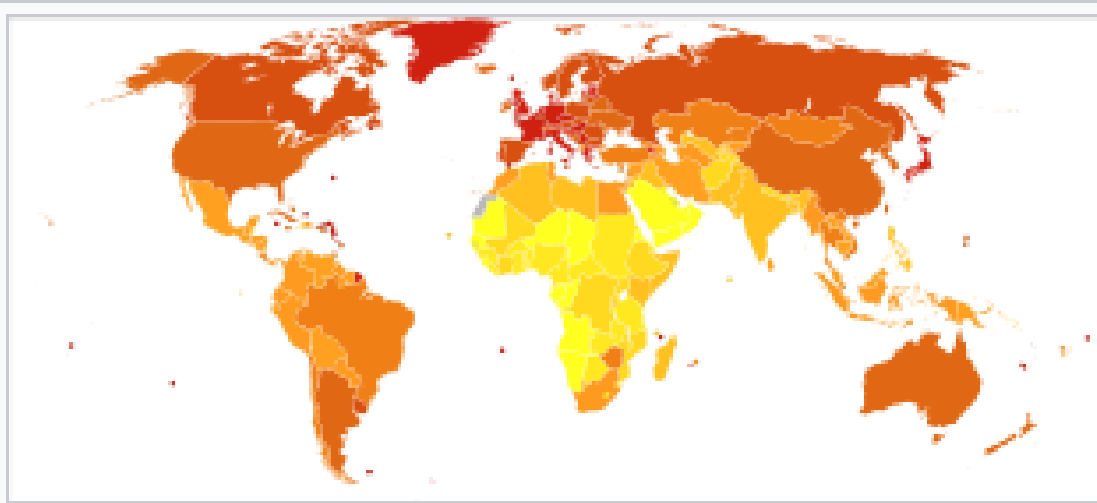SURAJ KHOCHARE AND XIAONAN SHAN

Outline:
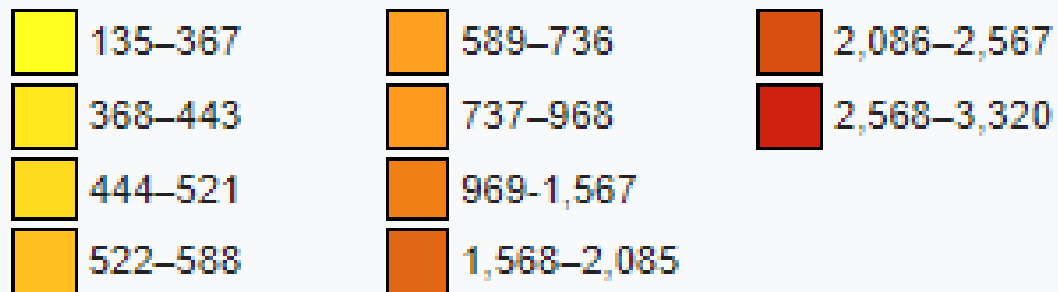
1. Background

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

Death from cancer per million persons in 2012

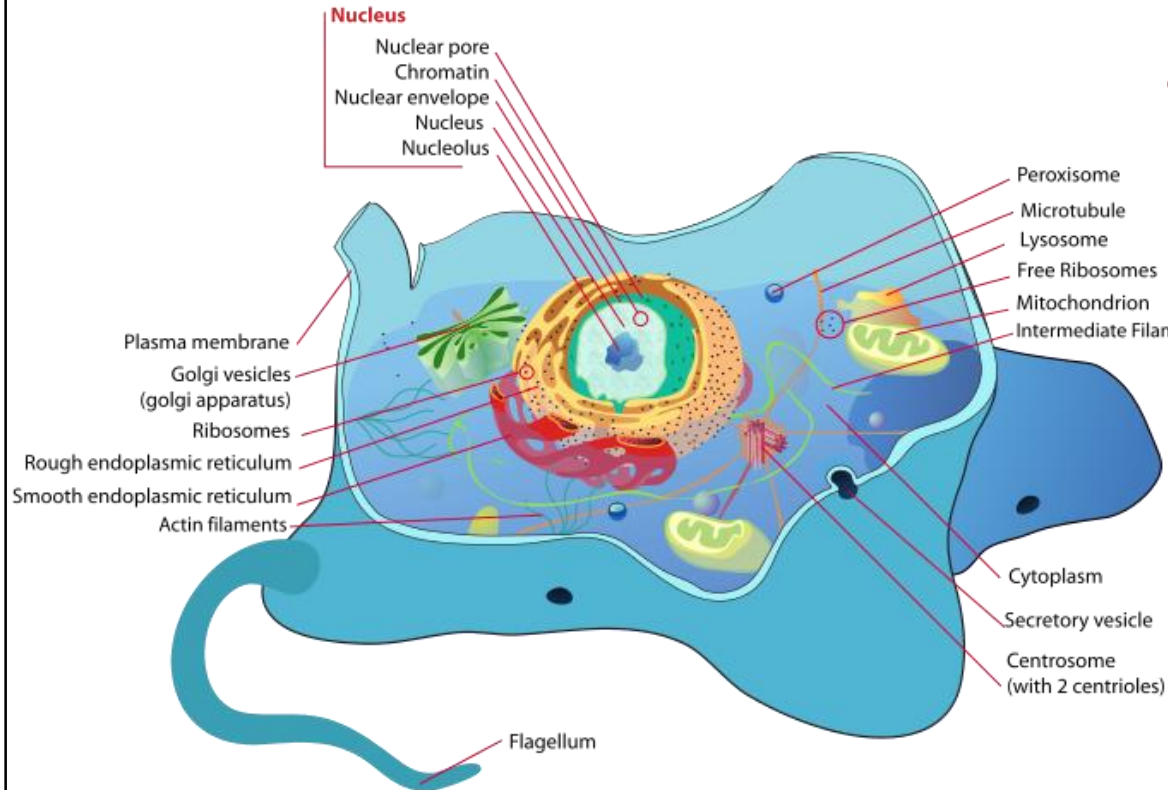|		
135–367	589–736	2,086–2,567
368–443	737–968	2,568–3,320
444–521	969–1,567	
522–588	1,568–2,085	

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).

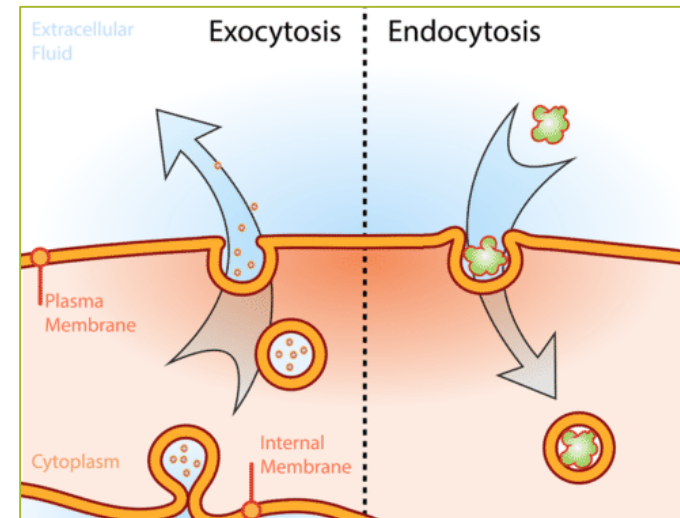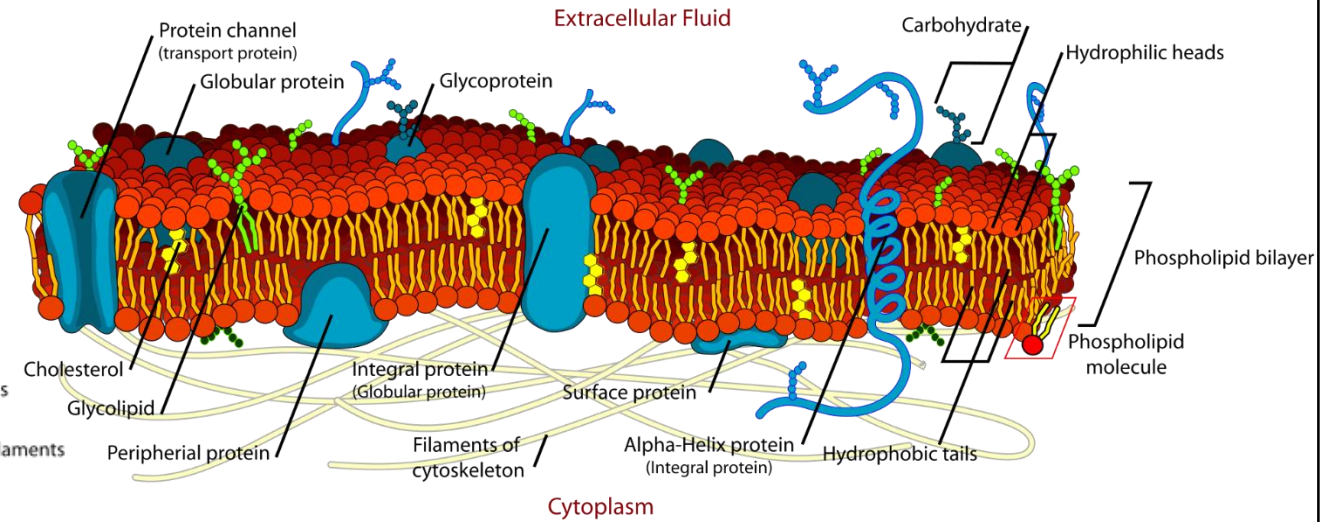Do we know the reason for cancer? | If not, can we study cancer cells? | Can we find a remedy?

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Introduction to Membrane Fluctuations:

Structure of a Biological Cell

Cause of Cell Membrane Fluctuations

Active fluctuations:

✓ Cross-membrane ion-transport.

✓ Cell structure

✓ Endocytosis and Exocytosis

✓ Live cells undergo continuous <u>active processes</u>.

✓ **Ex.** :- Metabolism, Metastasis, Mitosis, etc.
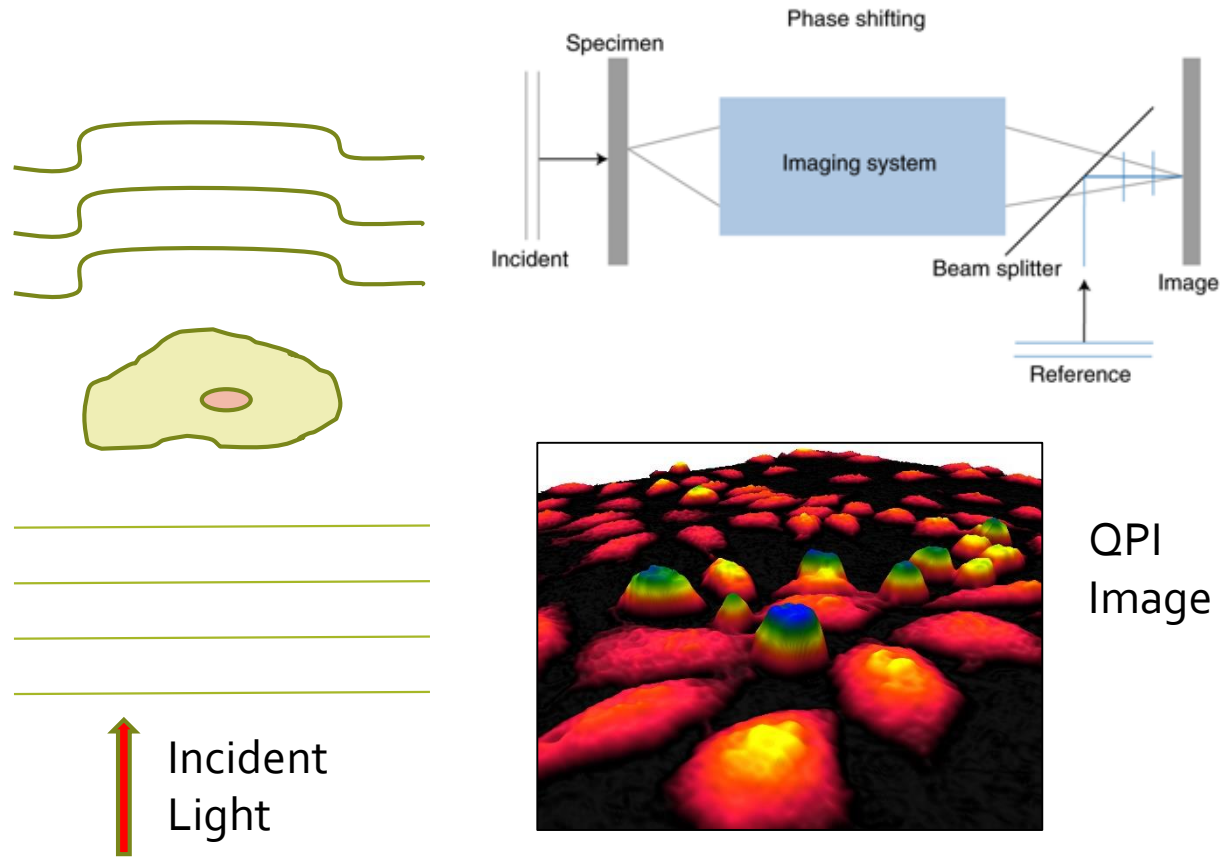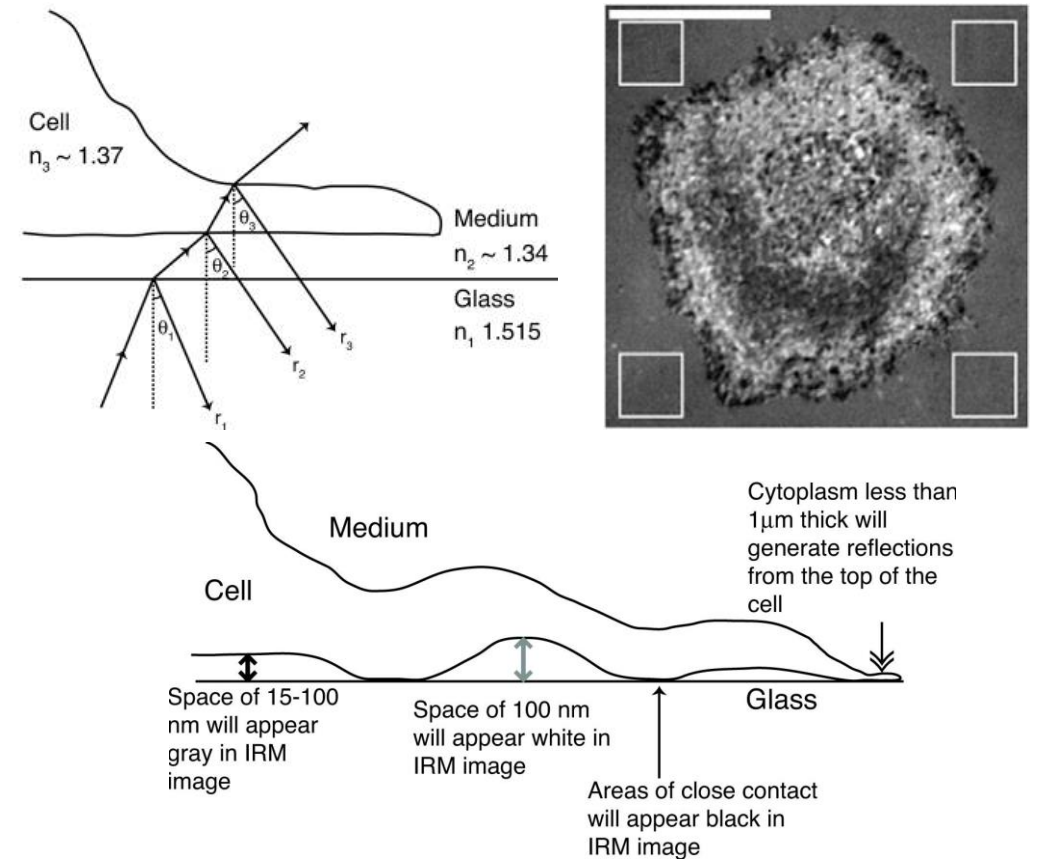
Techniques to Image Membrane Fluctuations:

Quantitative Phase Imaging

Interference Reflectance Microscopy

Our Approach: Surface Plasmon Resonance Imaging

Sensitive only to bottom membrane
- Image only the bottom cell membrane.
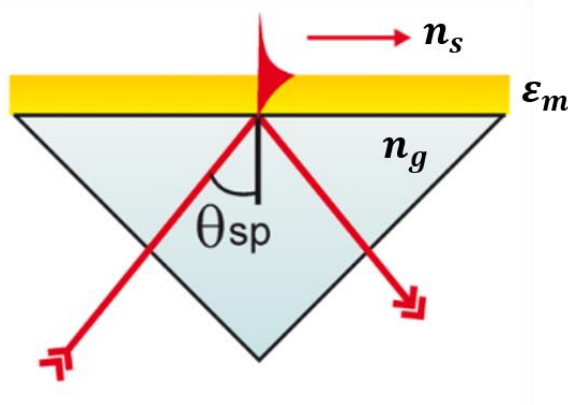- High sensitivity in z-direction, less distortion.

High Sensitivity : Sub-nanometer Membrane Fluctuations
- Study metabolism.
- Study metastasis.

Single Cells
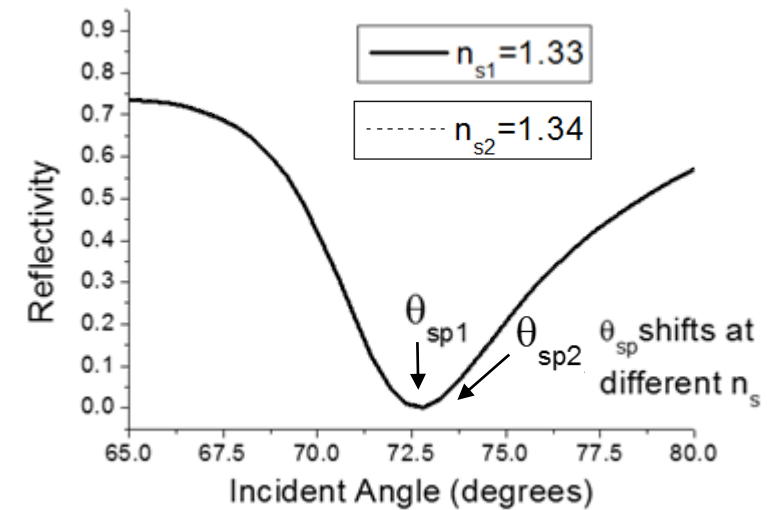- Study fundamental properties.
- Study cell heterogeneity.

Principle of Surface Plasmon Resonance (SPR) Imaging

✓ High Sensitivity

SPR Interface

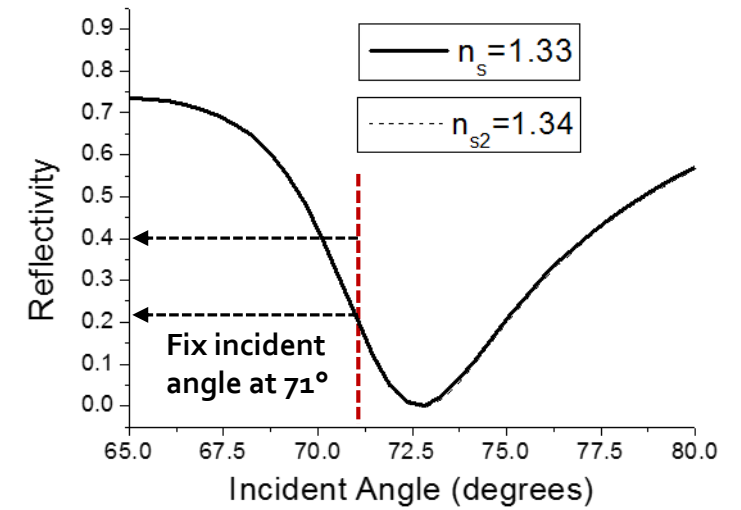$$\sin \theta_{sp} = \frac{\sqrt{\dfrac{\varepsilon_m n_s^2}{\varepsilon_m + n_s^2}}}{n_g}$$

SPR Angle

SPR Response

Live Cell Imaging Using SPR

Position at
o secs

Position at
't' secs

Fix incident
angle at 71°

$n_s = 1.33$

$n_{s2} = 1.34$

Δd nm
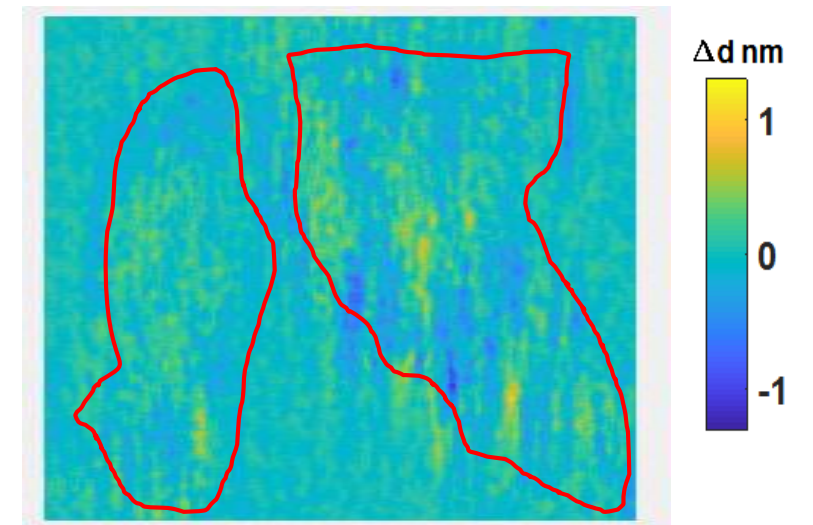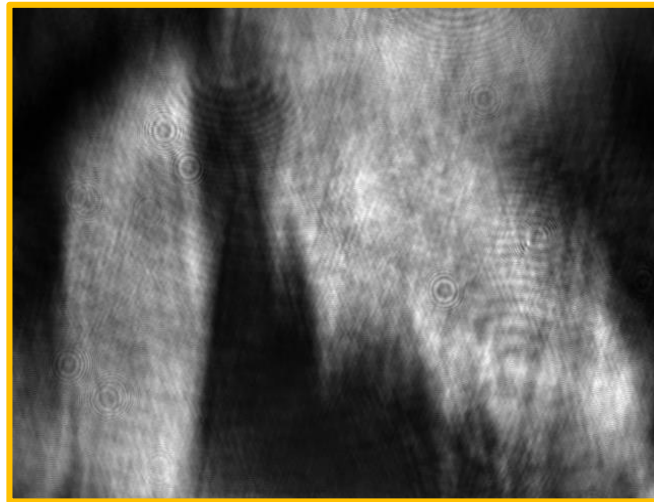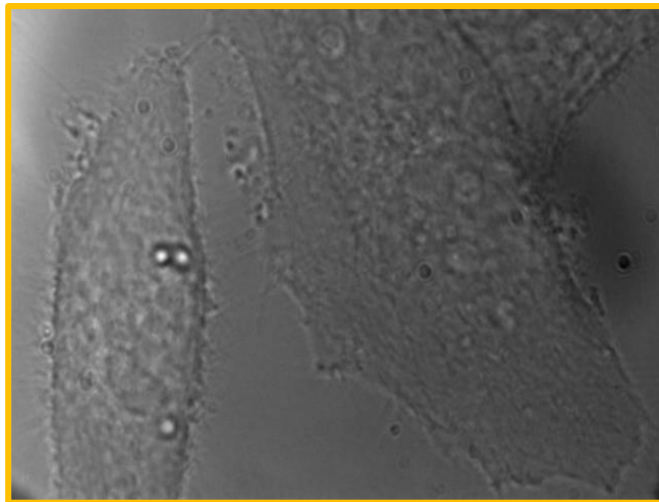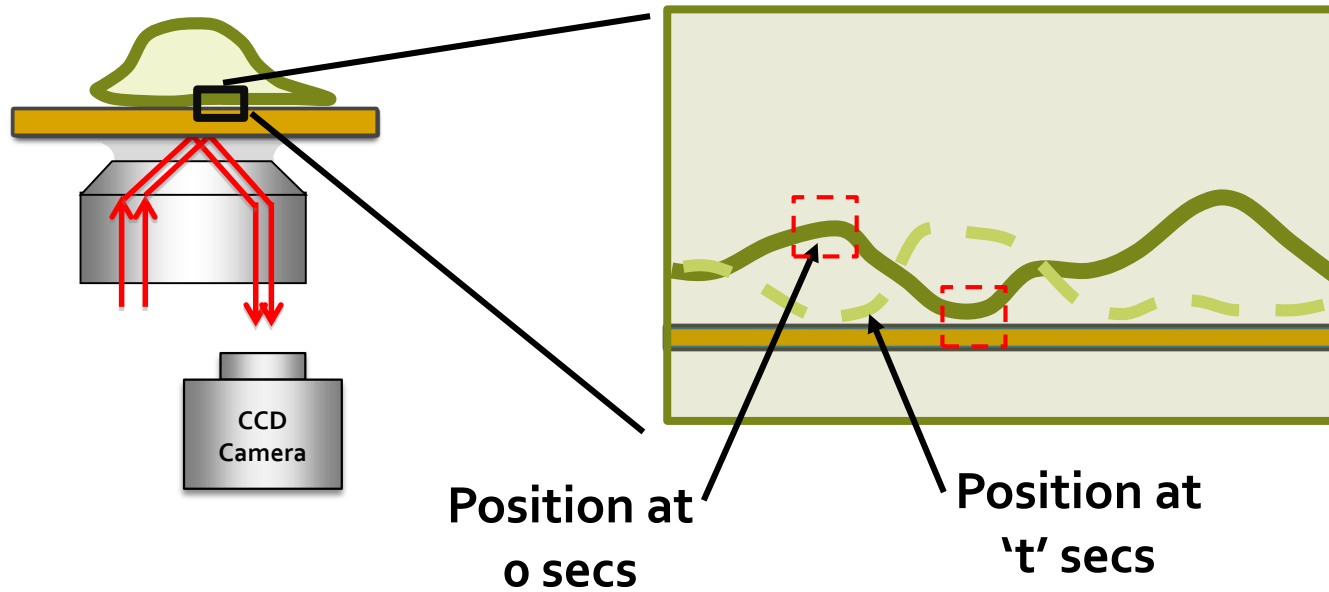
Mapping Reflected Light Intensity to Distance from Substrate
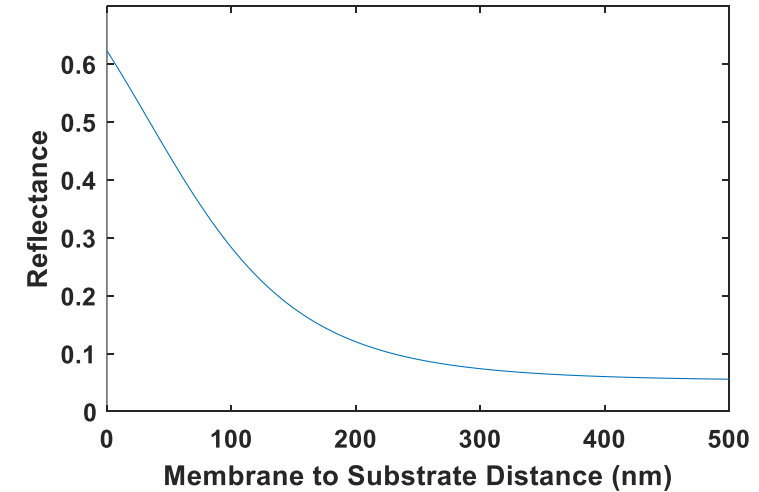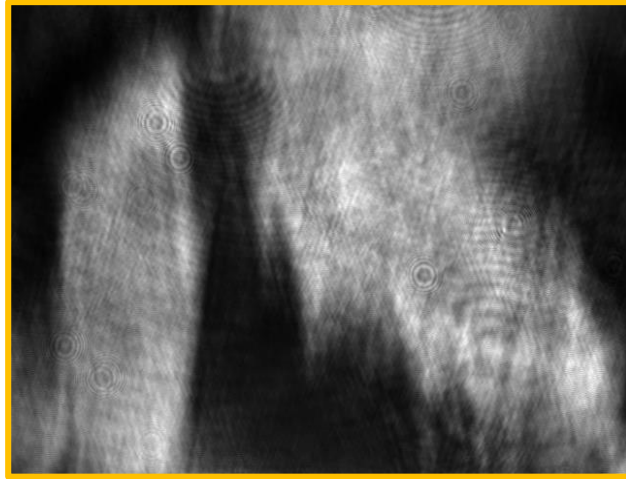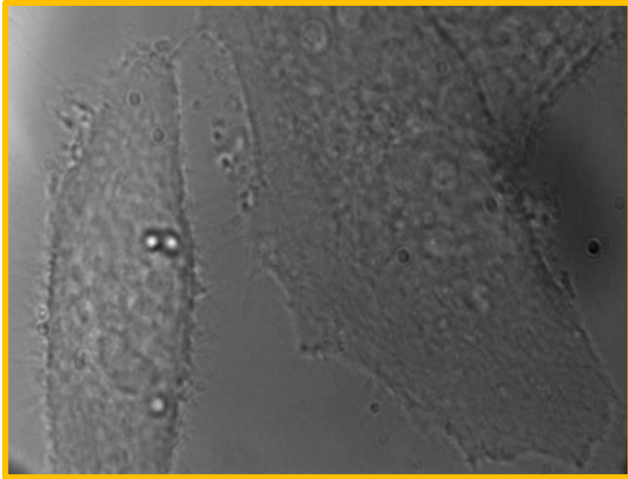
Calibration Curve

Simulation Model

|	
Layer 5	Cytoplasm
Layer 4	Cell Membrane
Layer 3	Culture Medium
Layer 2	Au
Layer 1	BK7
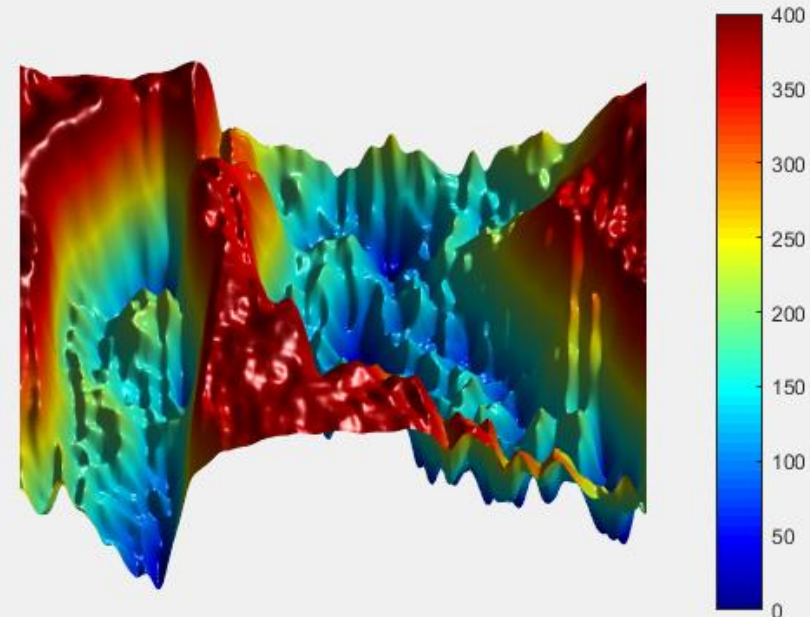
Analyse Sub-Nanometer Membrane Fluctuations

a) t = 5 sec

b)

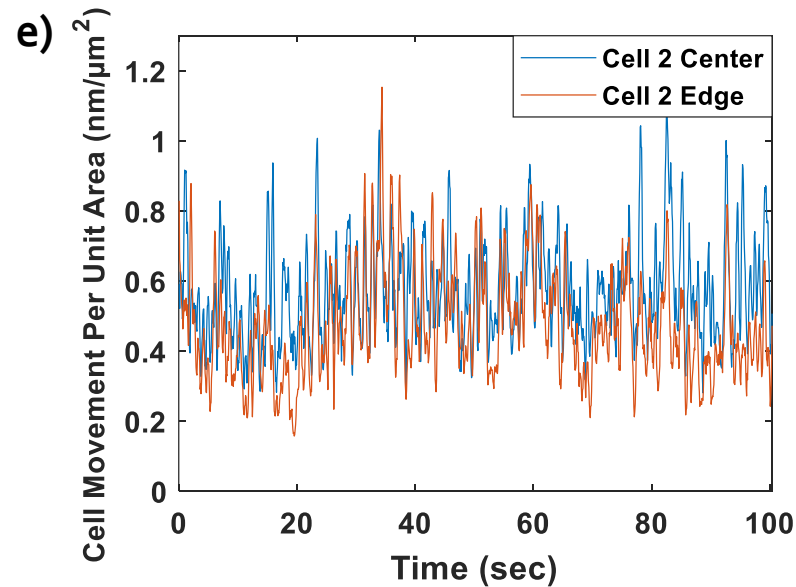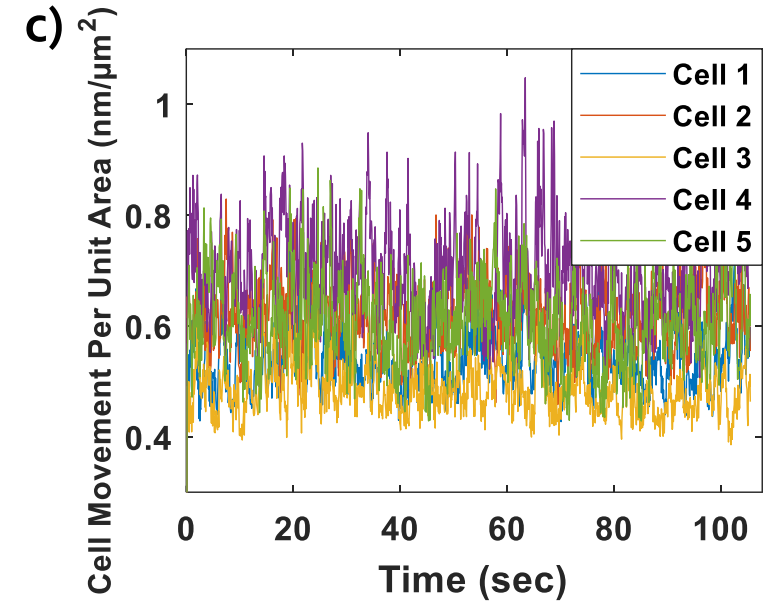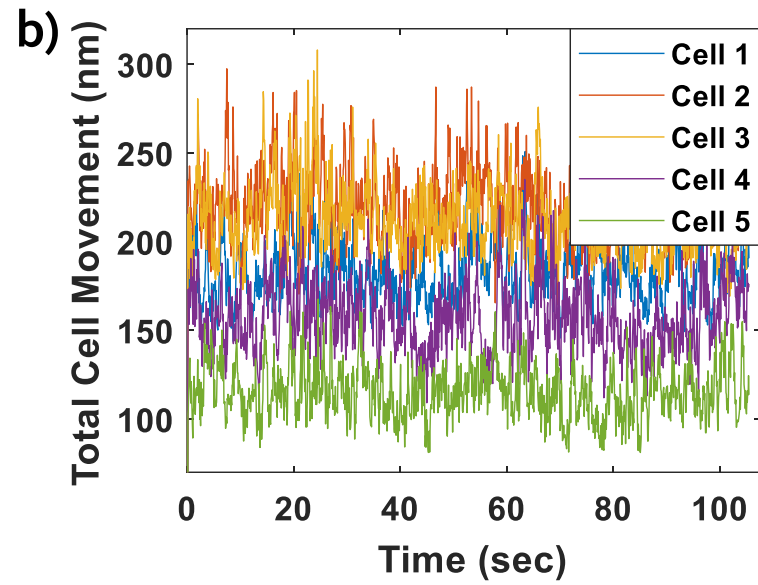c) t = 6 sec

d) t = 6.5 sec

Scale Bar = 10 μm
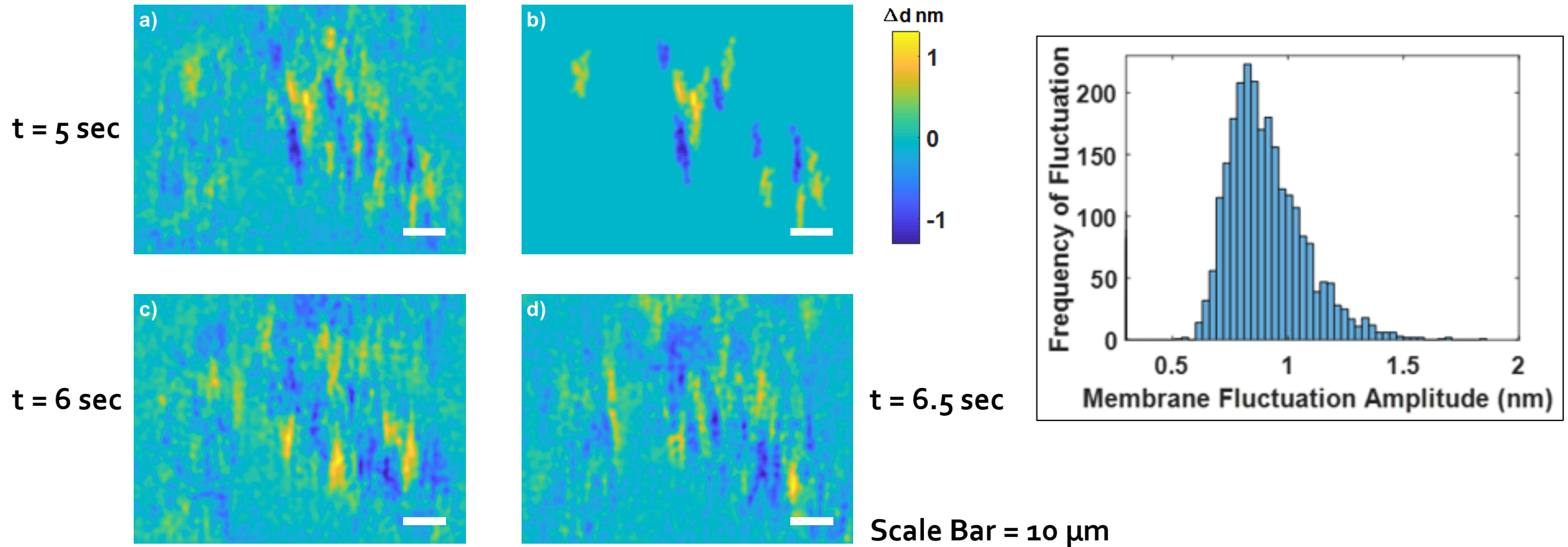
✓ Image small yet swift movement.

✓ Distribution range for fluctuations.

✓ These movements may contribute to respiration, metabolism, active fluctuations and thermal fluctuations.

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Quick Review
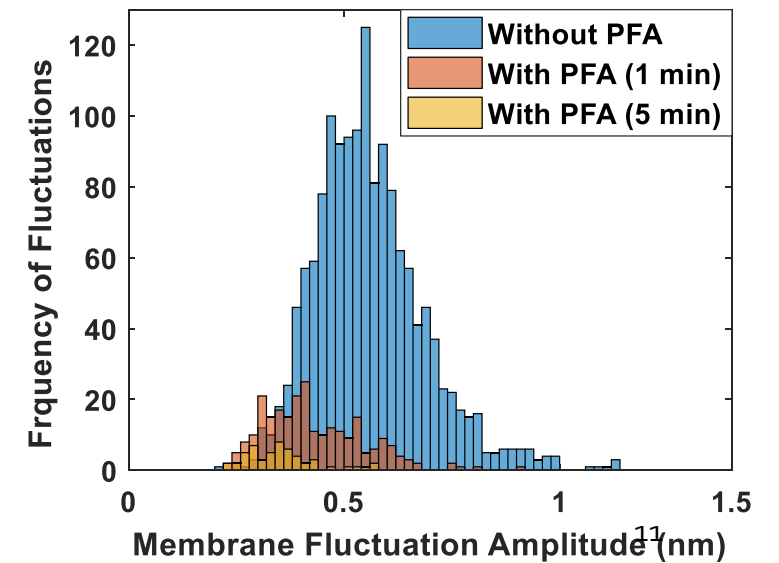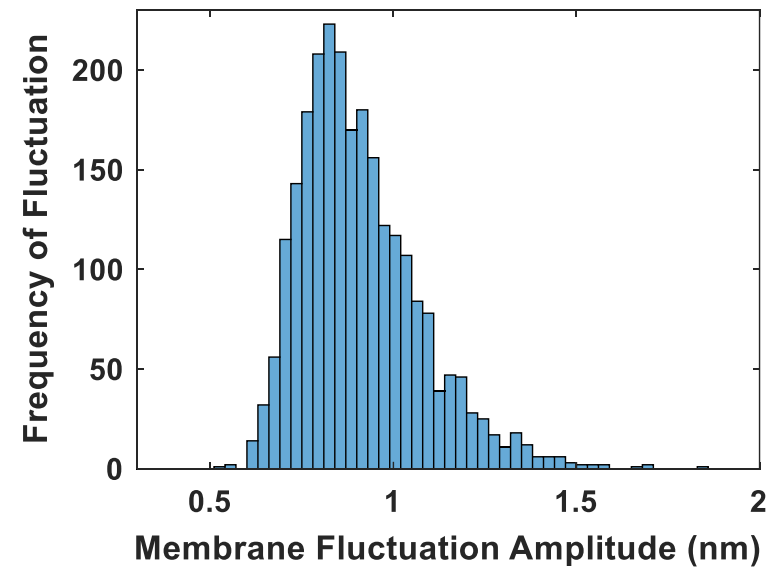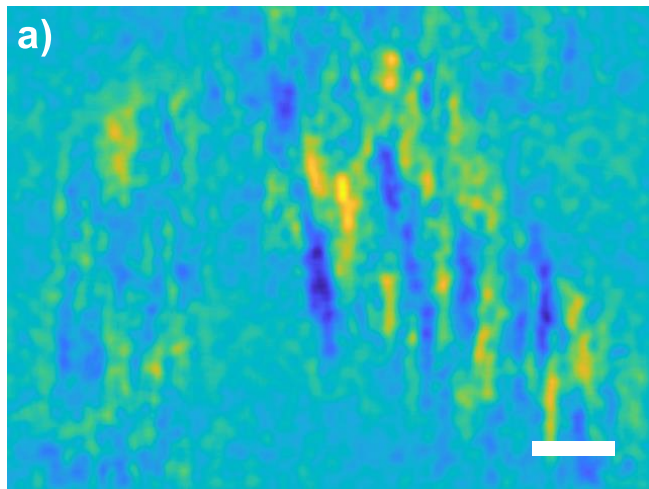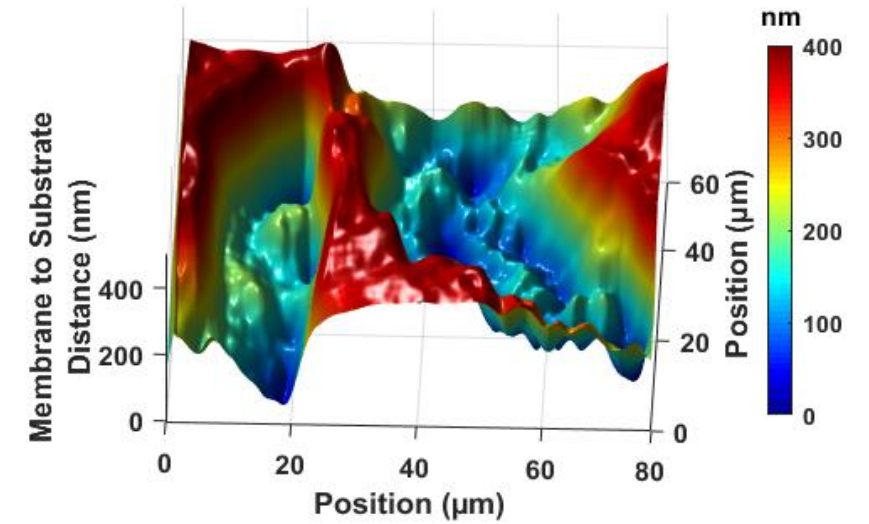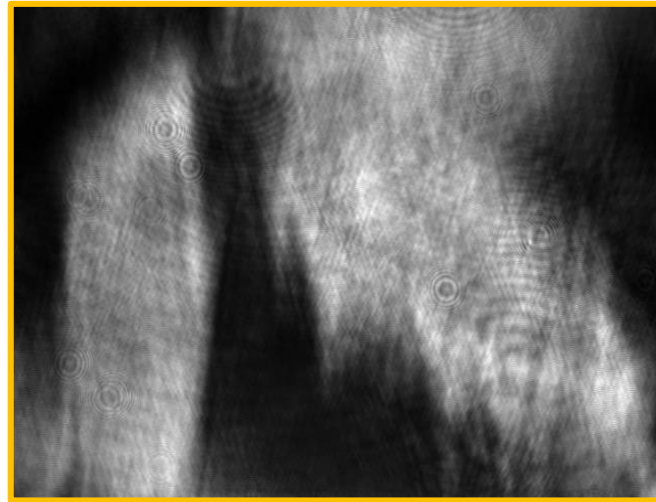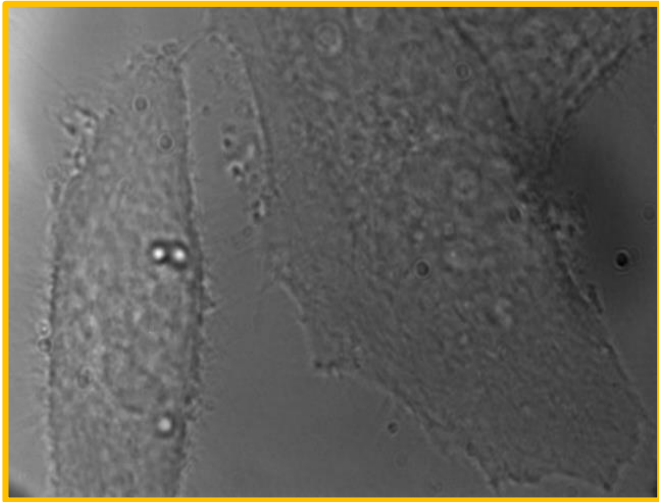
Cell Heterogeneity
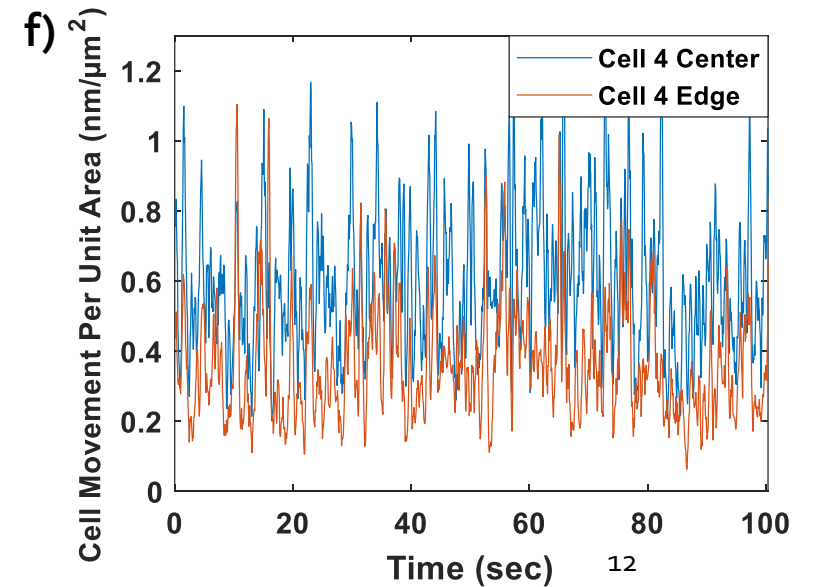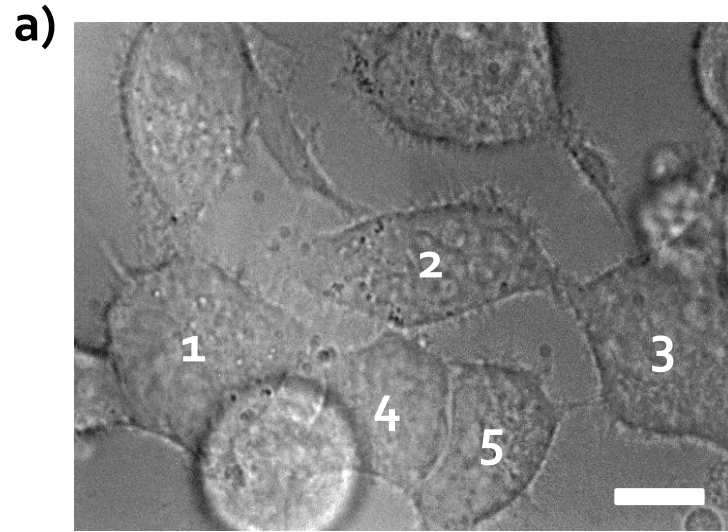
Long Duration Cell Membrane Movement

(Scale Bar = 10 μm)

3D Video Displaying Membrane Movement
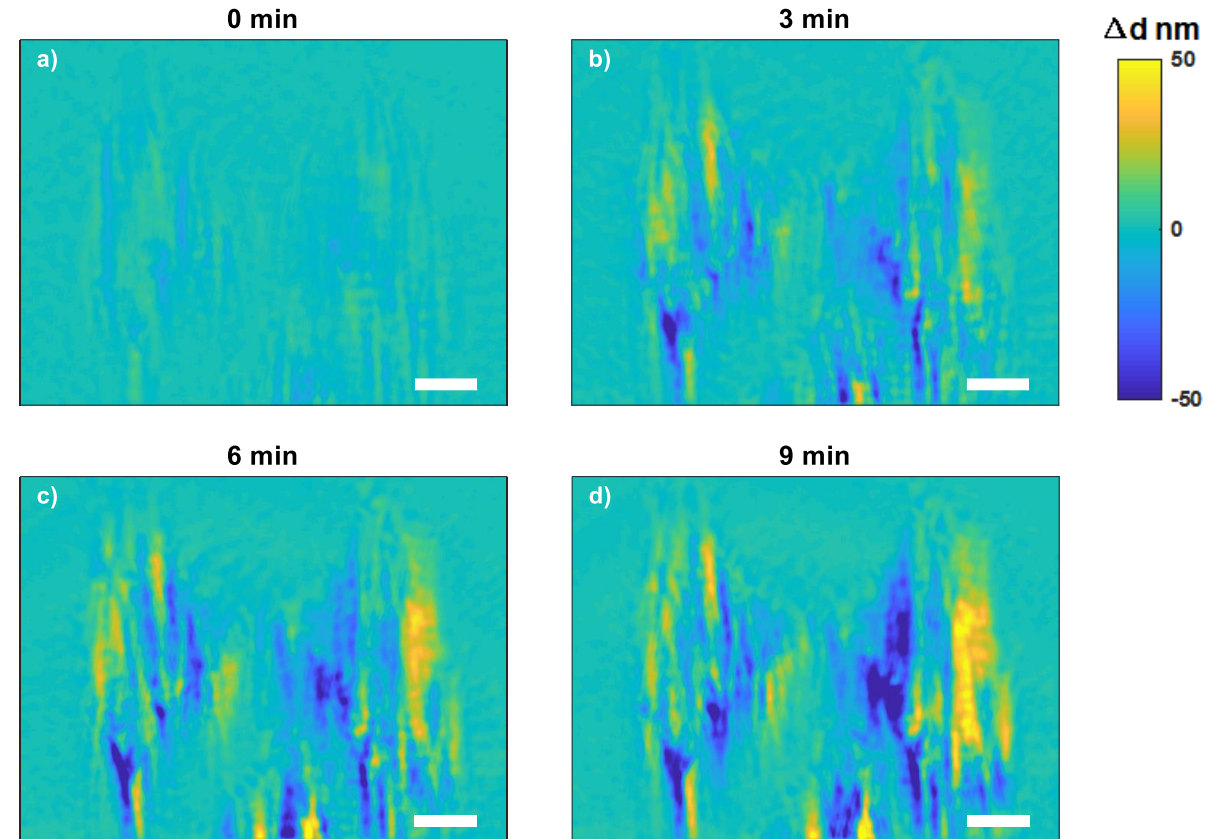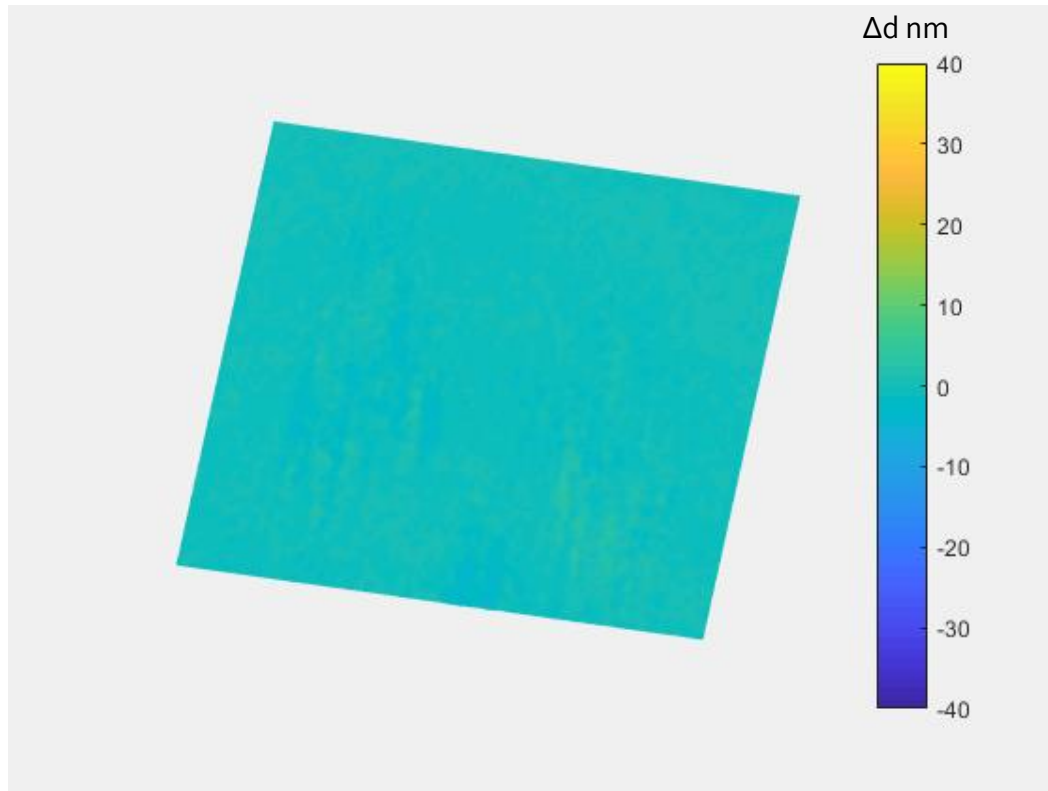
✓ We see a <u>collective movement</u> (10's of nanometers) as we record for longer time.

✓ This can be related to <u>physiological processes</u> like metastasis.

✓ Do they have a correlation with the sub-nanometer membrane fluctuations?

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Cell Metastasis

HT-1080
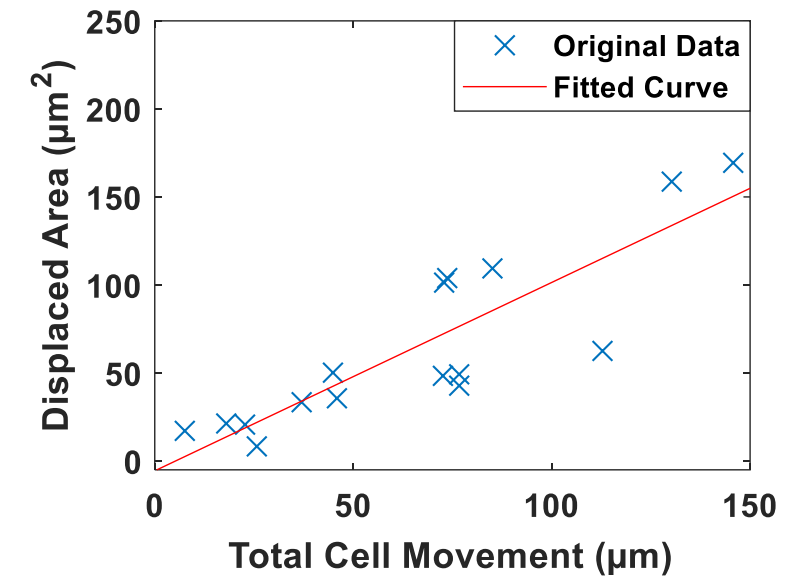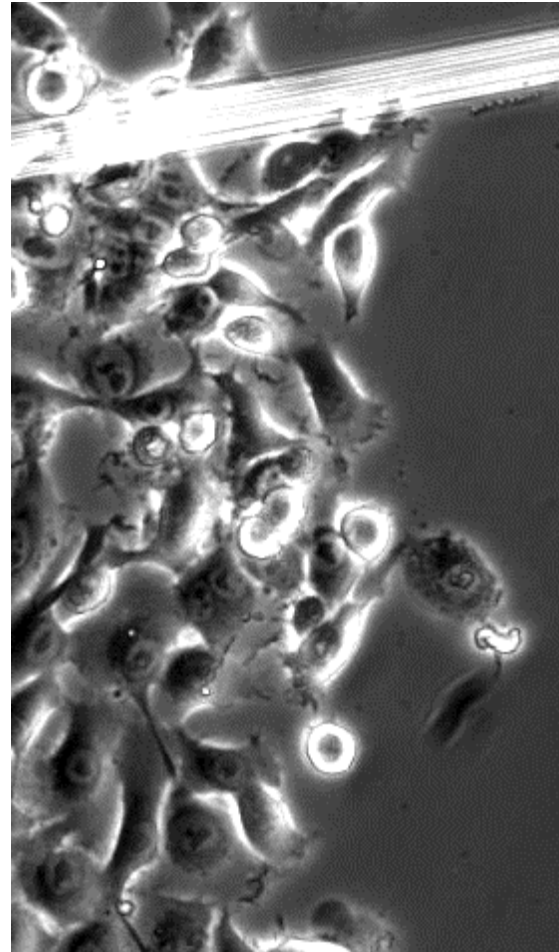
Displaced Area α Membrane Movement
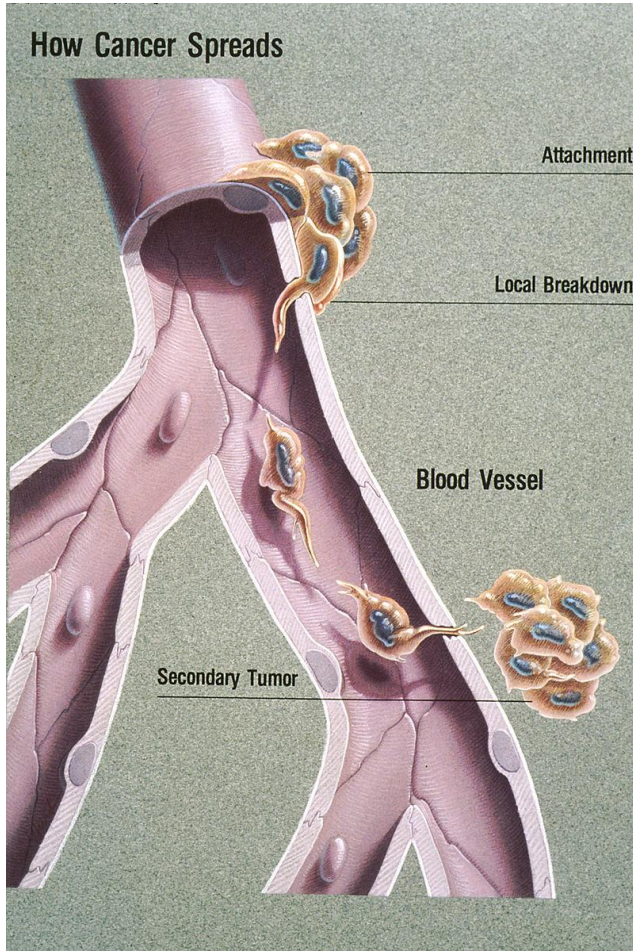
Conclusion:

✓ 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.
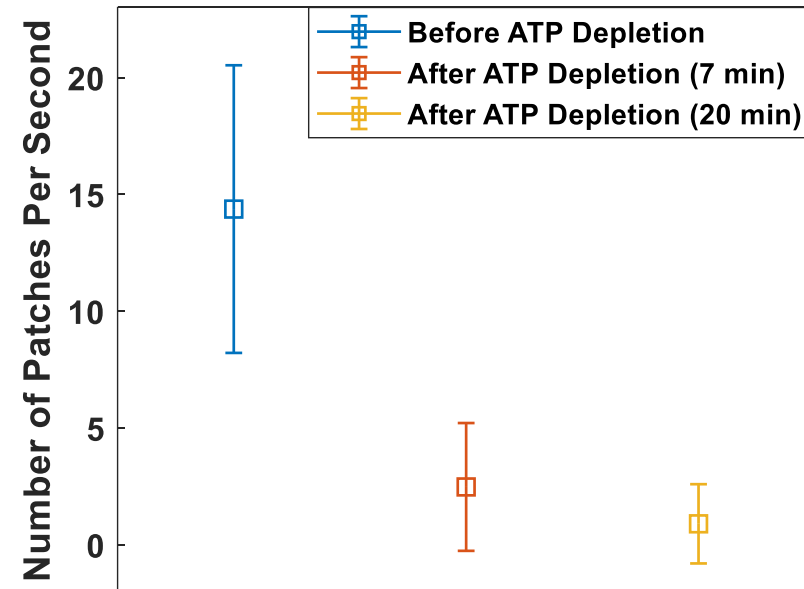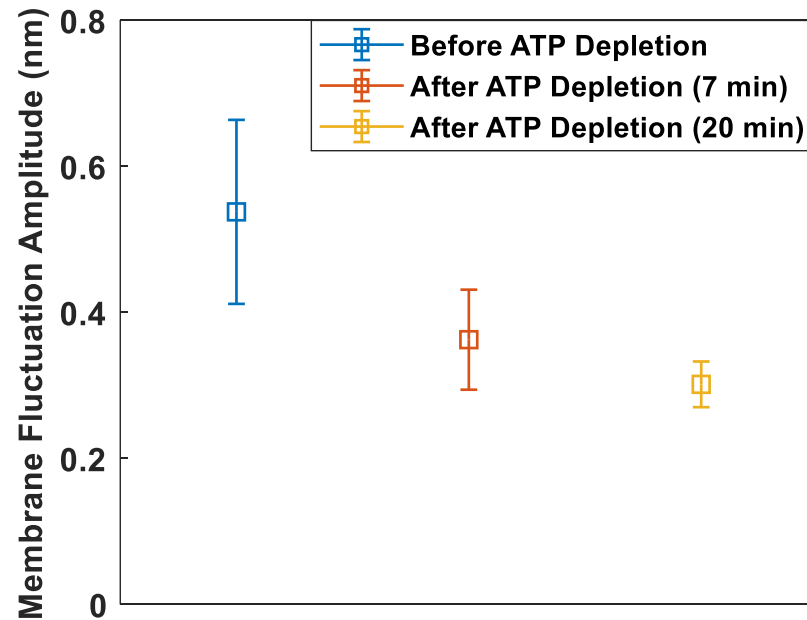
Complementary and Non-complementary DNA Binding Analysis

5'   3'

TTG TGT CCA GGG TGT GTT

3'   5'

                                                    Jones ssA SH

-S-T$_{10}$-AAC ACA GGT CCC ACA CAA-A-AGAGA

                              TCTCT-A-AAG AGA CCT GCA ACA CAA-T$_{10}$-S-
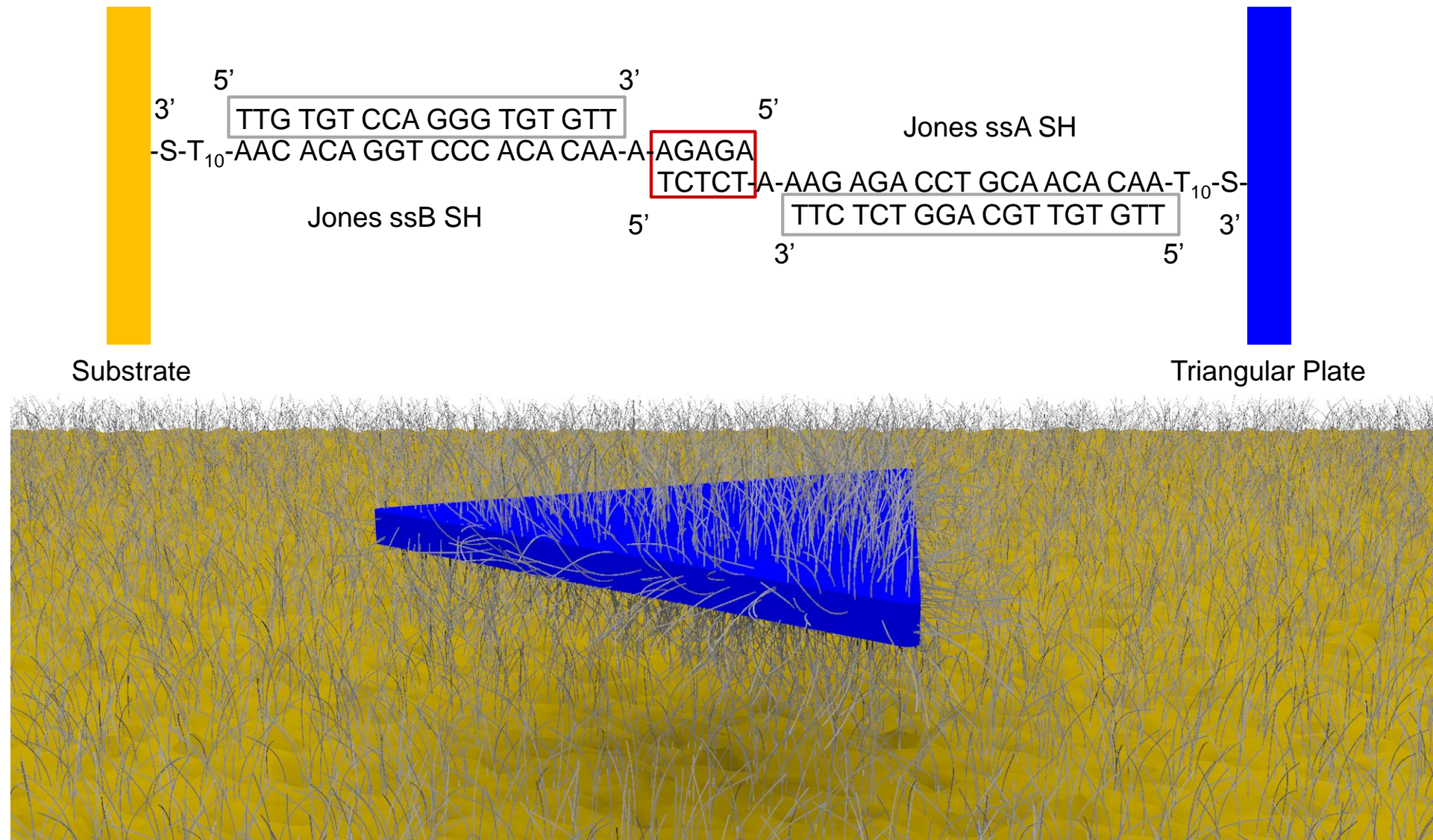
Jones ssB SH

                                    5'   TTC TCT GGA CGT TGT GTT   3'

                              3'   5'

Substrate   Triangular Plate
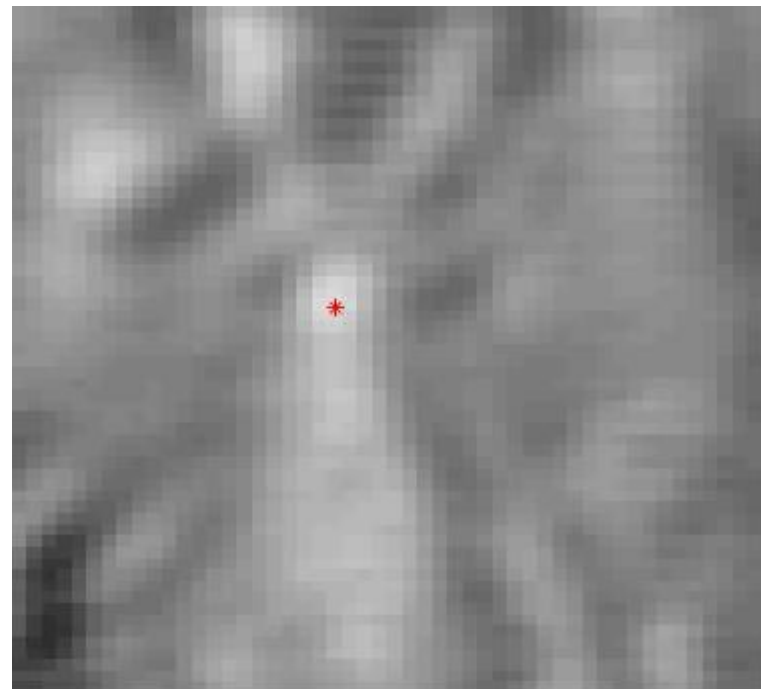
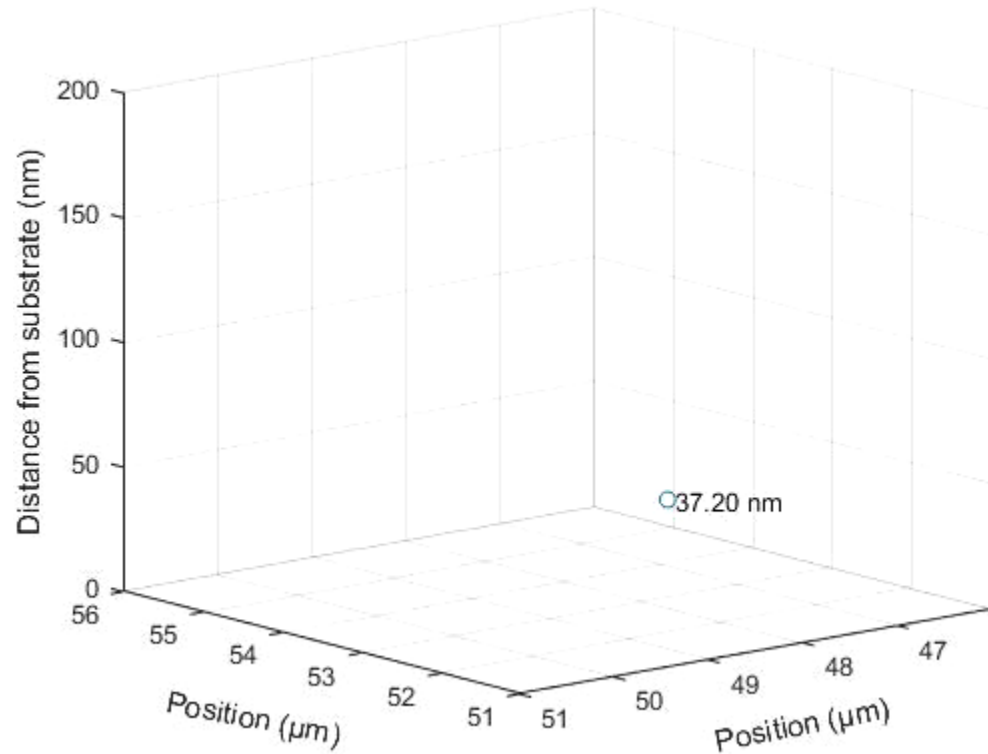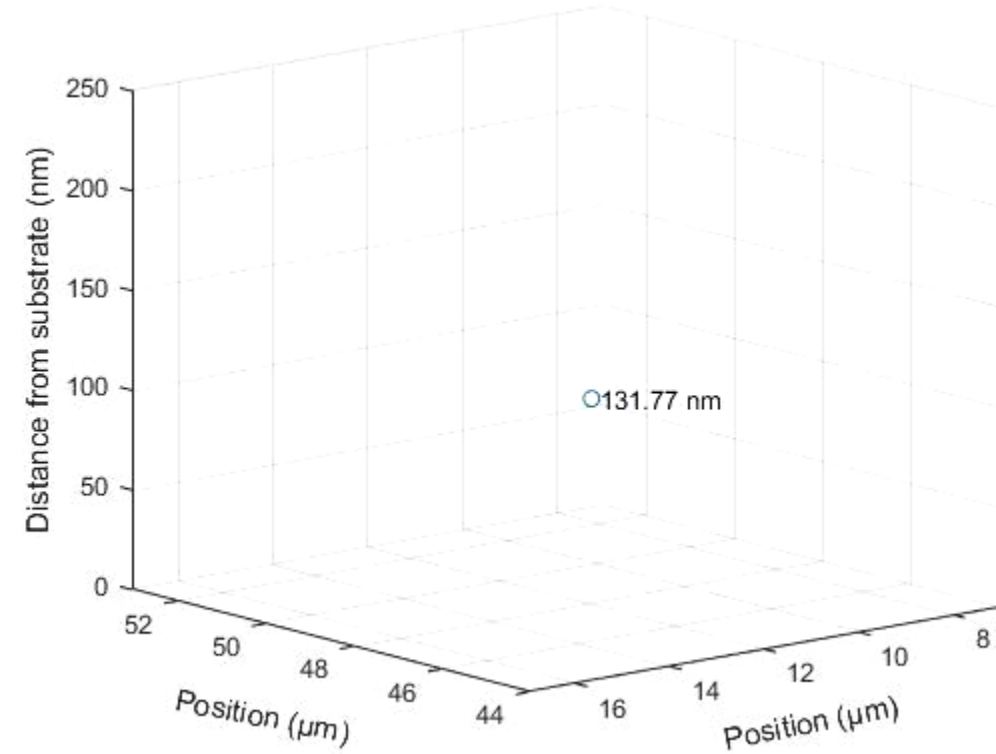❖ **Study DNA Binding using SPR Imaging:**

Original Image

Cropped Image

Complementary Binding

Non-Complementary Binding

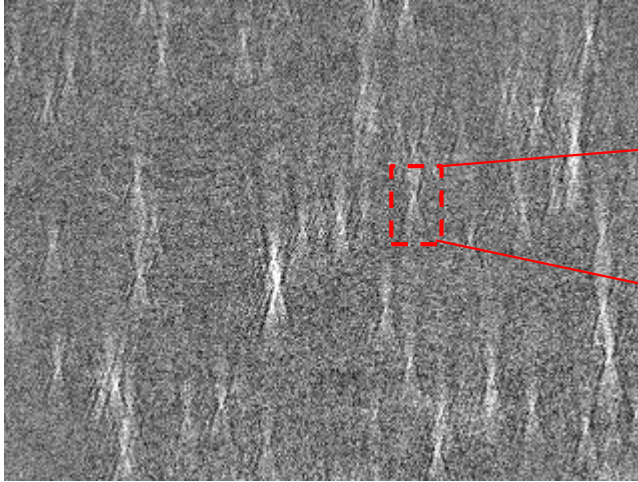**Conclusion:**
✓ **Mean-square displacement:** Non-complementary ~ 10 * Complementary
✓ **Diffusion Co-efficient:** Non-complementary ~ 100 * Complementary

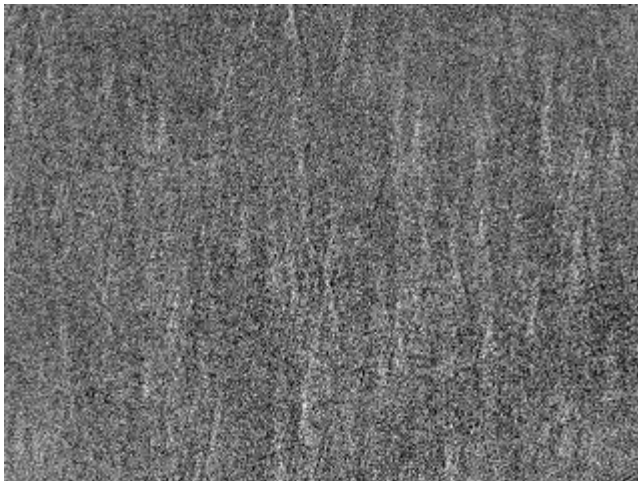# Automated Detection of Low-SNR SPR Signals using Machine Learning

P₃

PSNP 65 nm

PSNP 42 nm

✓ **Contrast**   ✓ **Position**   ✓ **Efficiency**

✓ **Resolving Power**

❖ **Videos for Particle Binding to the Plasmonic Substrate**

65 nm PSNP

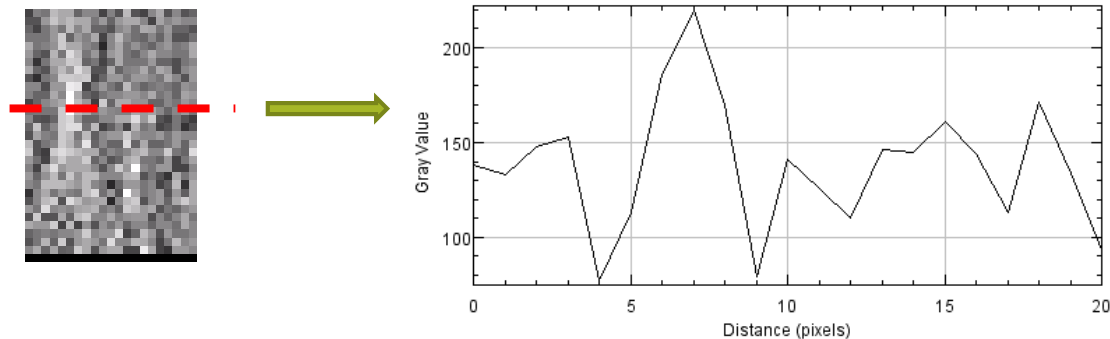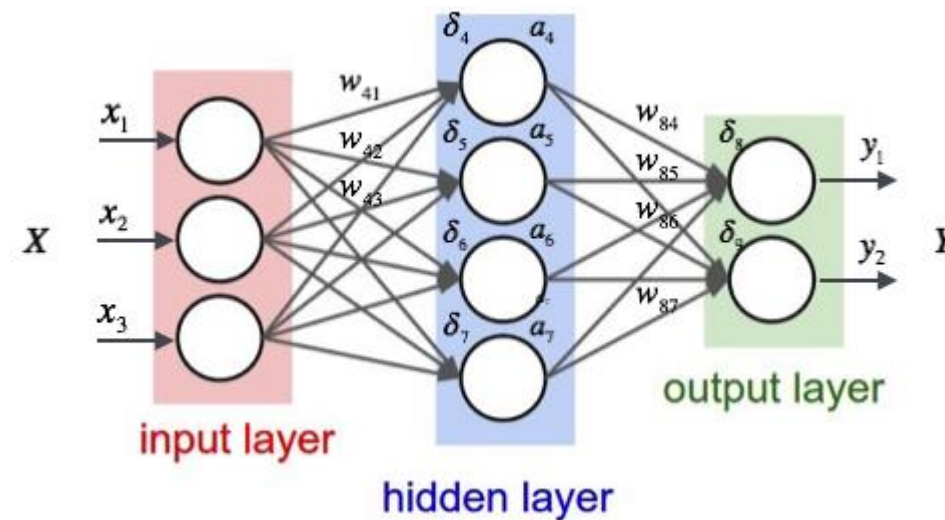**42 nm PSNP**

✓ Low signal-to-noise ratio.

✓ Hard to evaluate using image processing algorithms.

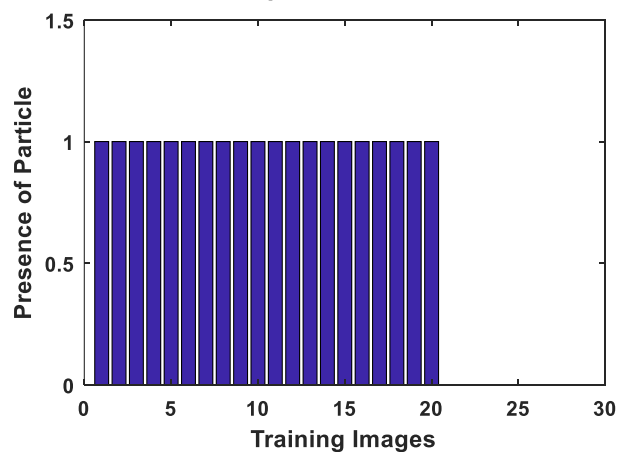# ❖ Train Neural Network for 65nm PS nano-particles
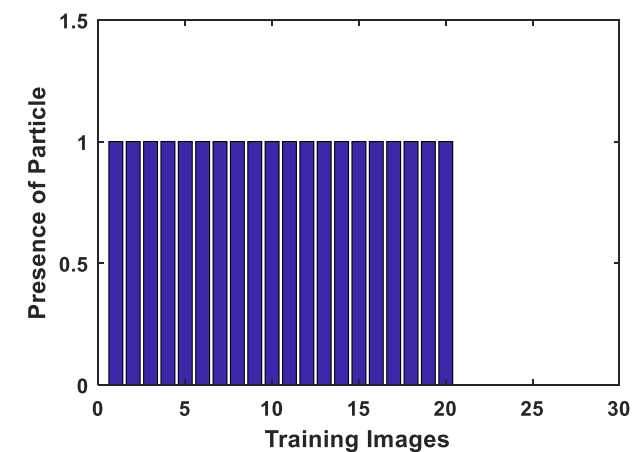
Input Images

Training Network

input layer

hidden layer

output layer

Accuracy 100%

Input Data

Testing Data
(Training Data)

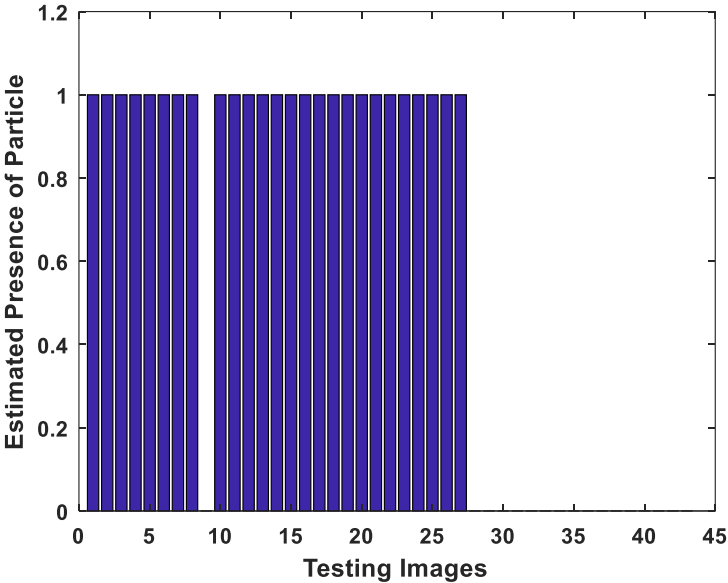Train Network

Weights

Result

❖ **Results for test images (1000 iterations):**

❖ **Results:**

Test Image (F67)   Result Test Image (F67)
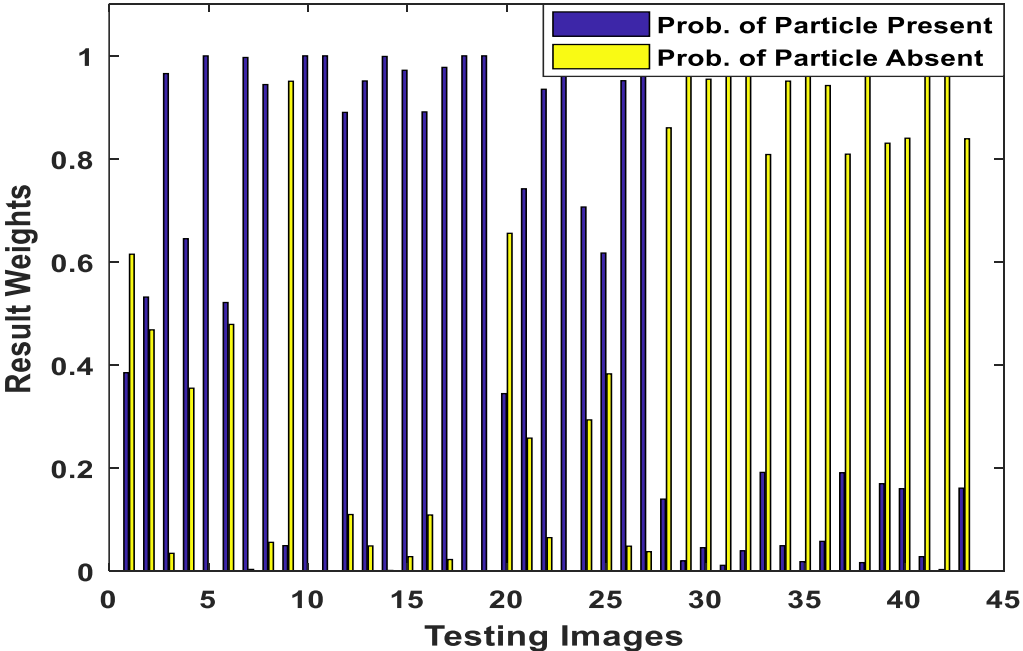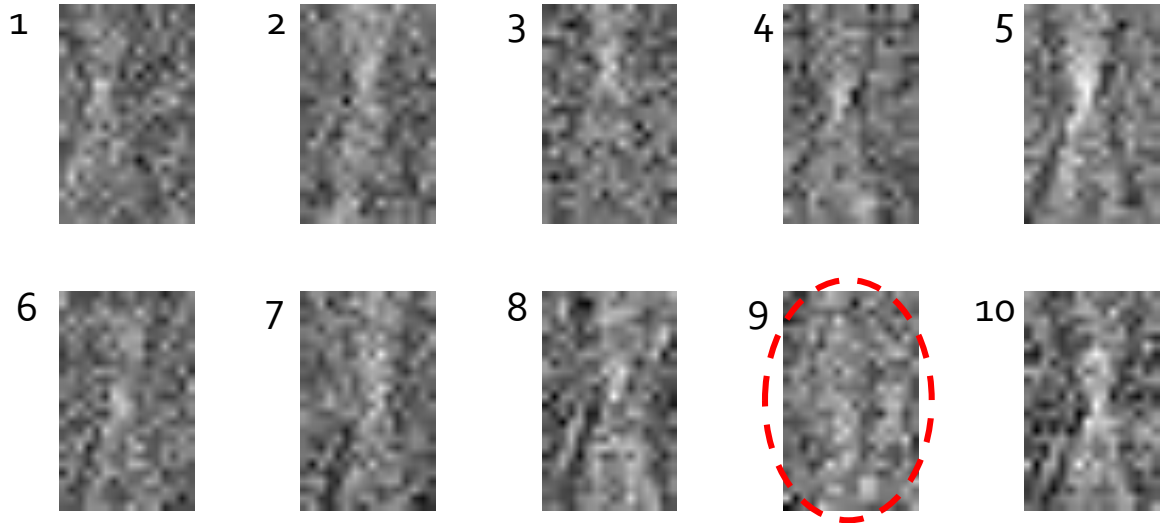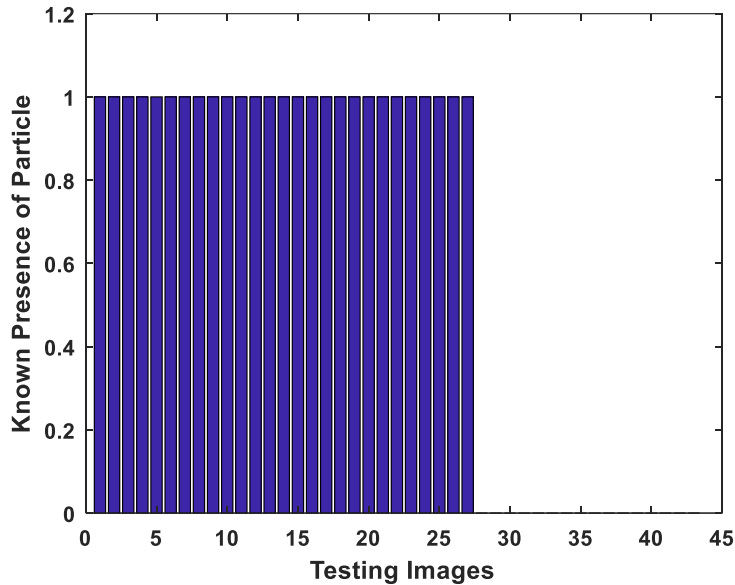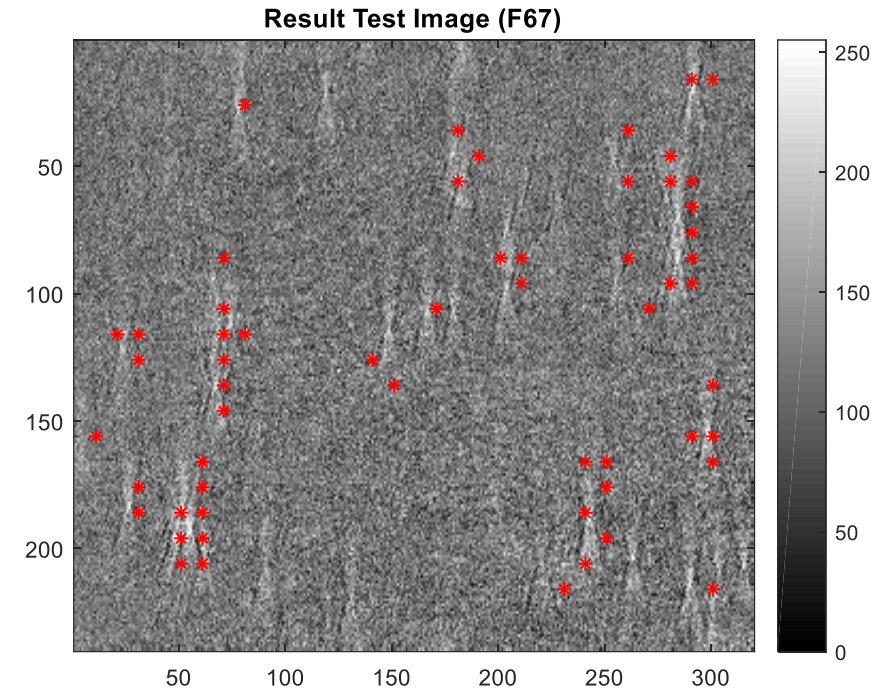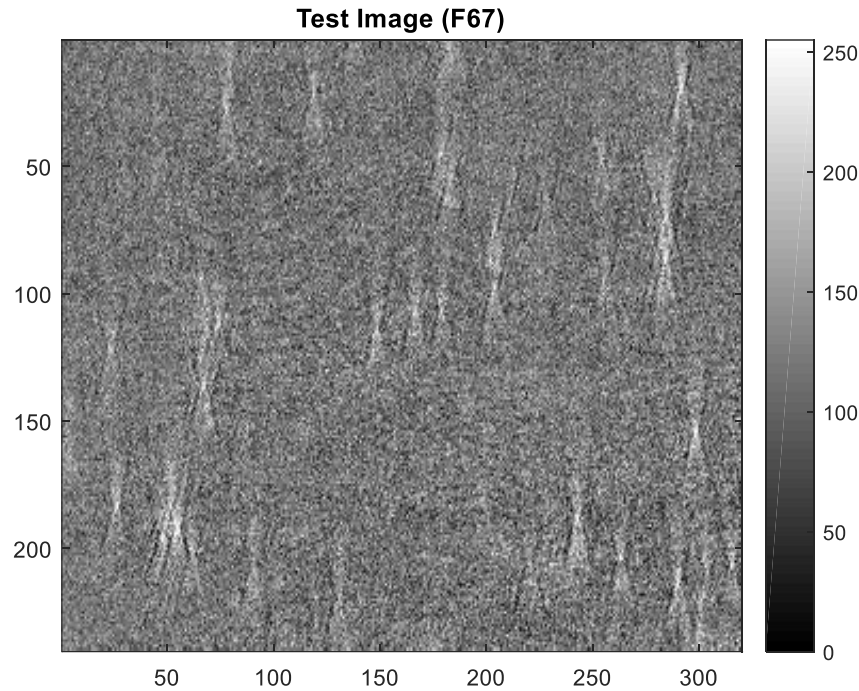
❖ **Conclusion:**

✓ Increasing number of hidden layers improves signal detection.

✓ Increasing training dataset improves network training.

✓ Increasing number of iterations helps improve detection accuracy.

Image Segmentation To Quantify Cloud Coverage In Night All-Sky Camera Images

Indian Institute of Astrophysics, Bangalore

Objectives:

- Quantify cloud coverage at a given geographical location.

- Deliver software tool to help astronomers in site characterization.

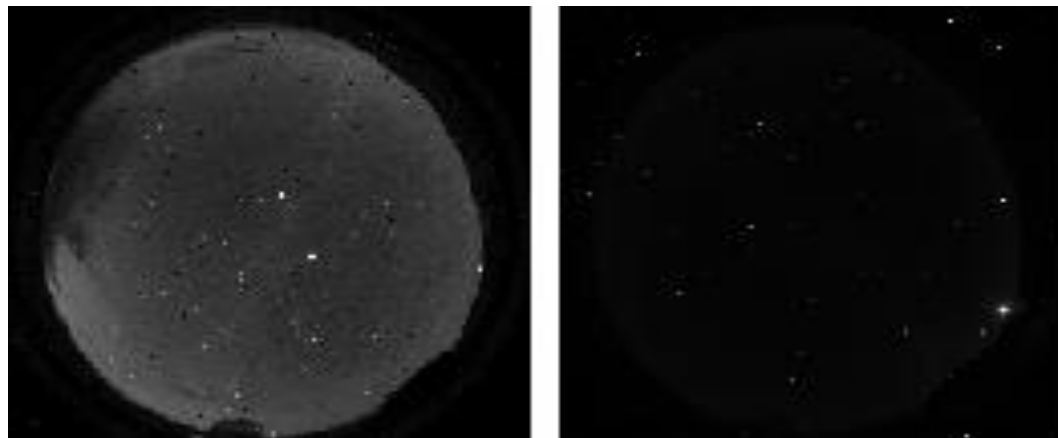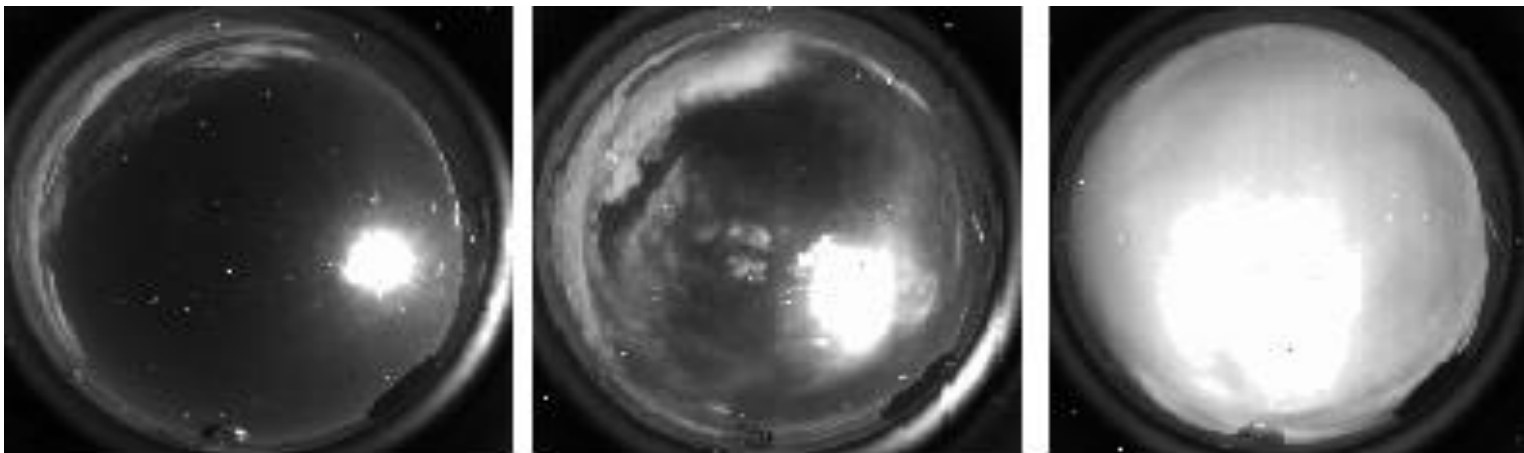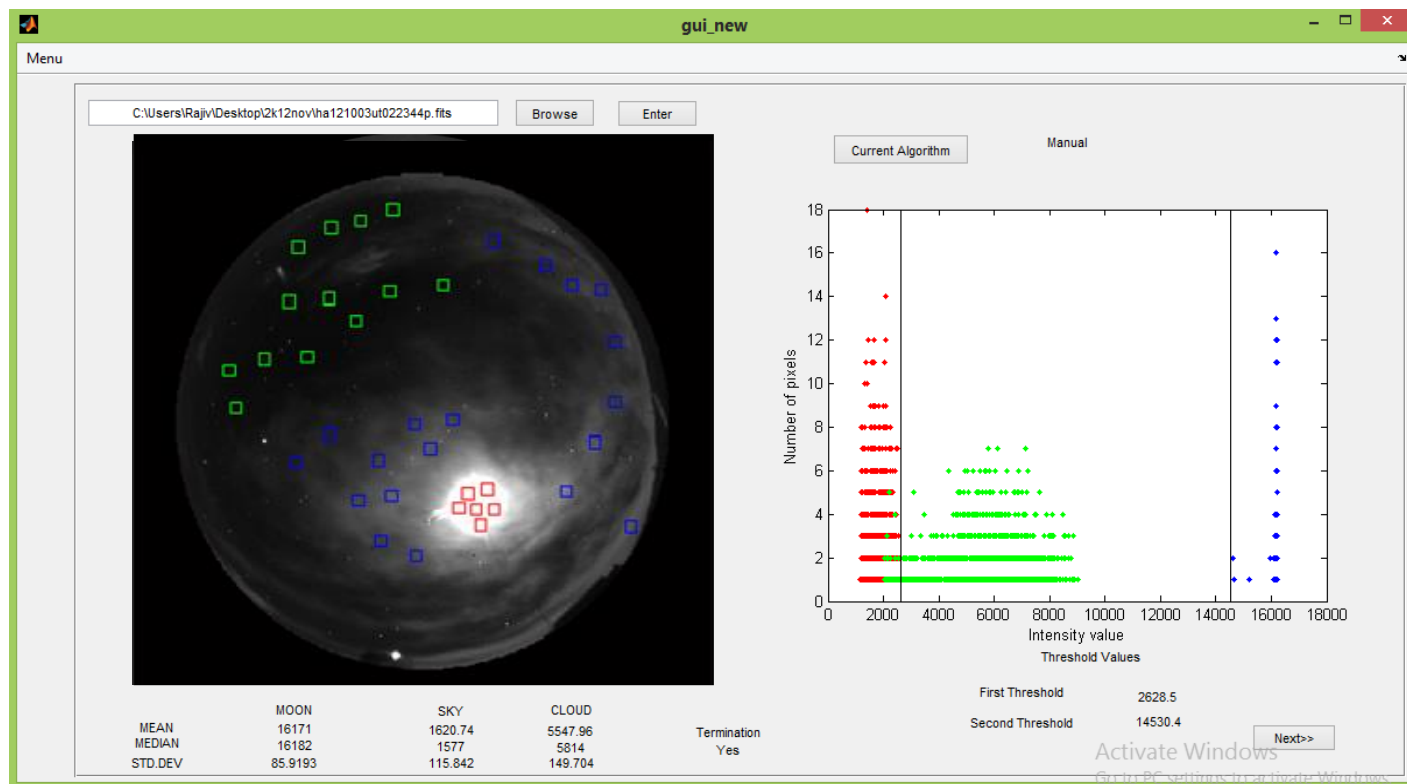# ❖ User Interface and Statistics of the Night Sky Regions:

Results

	Sky	Cloud	Moon
Case 1	Present	Present	Present
Case 2	Present	Absent	Present
Case 3	Absent	Present	Present
Case 4	Present	Present	Absent
Case 5	Absent	Present	Absent
Case 6	Present	Absent	Absent
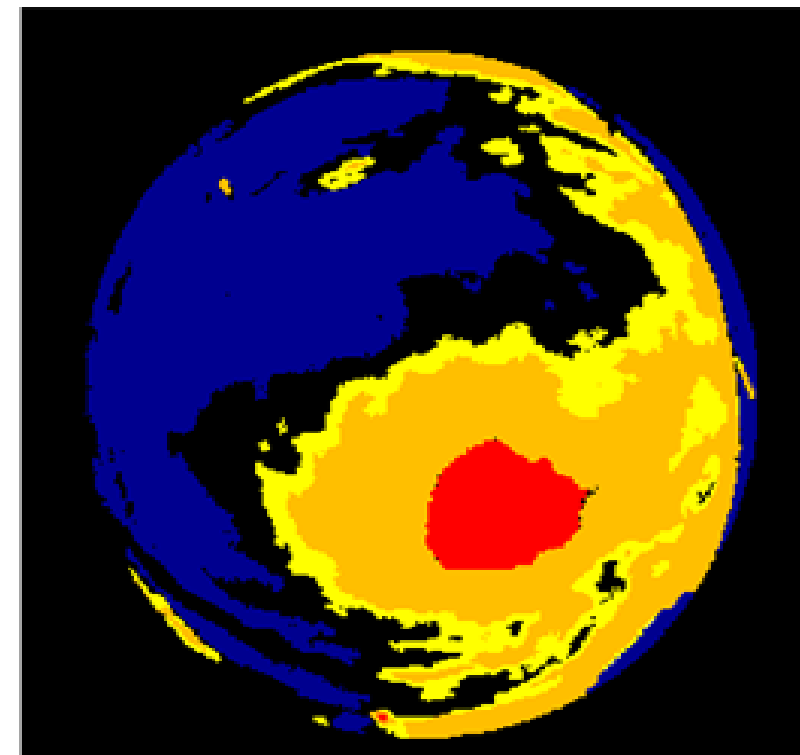
- ✓ **Matlab GUI.**
- ✓ **Choice of 3 algorithms** (manual and automated segmentation).
- ✓ **Statistics of the night-sky.**

Conclusion:

- ✓ Quantitative analysis of swift and tiny membrane fluctuations at a single cell level help us to analyse some fundamental properties of cells.

- ✓ Addition of z-direction in image analysis improves the desired results.

- ✓ Machine learning can provide us with solutions to problems which traditional image processing algorithms find difficult to deal with.

- ✓ User-friendly software tools with semi-automated and/or automated features enables necessary manipulation of procedures along with reduced workload.

Questions..?

Thank You !