Imaging the Sub-nanometer Membrane Fluctuations in Single Cells Using a Plasmonic Imaging Microscope

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Significance

- Plasma membrane of cells is undergoing continuous motion, part of which is driven by the active processes while the other part is purely thermal.
- This membrane fluctuations are highly relevant for biological system and many physiological processes, including fusion of liposome vehicles, cell division and cell's mechanoresponse.
- The membrane fluctuations also provide us with the inside information of viscoelastic properties of the cells.
- However, it is difficult to quantify the cell membrane fluctuations experimentally due to its small movement.
- Quantitative analysis of these mechanical vibrations provide us with mechanical properties of the cell membrane, information about the cell structure and metabolism in the cancer cells.
- This analysis is very essential to analyze activity of biomarkers when cancer cells are exposed to different drug treatments.

Innovation and Approaches

- We have used the plasmonic microscope to image the cell membrane fluctuations at single cell level. A 1-2 nm cell membrane vibration has been imaged with 0.5 um spatial resolution in real time.

Working Principle

The Surface Plasmon Resonance (SPR) Microscope setup is similar to Total Internal Reflection Microscope except that there is a 43 nm metal coating on the surface of SPR substrate. We use gold as a lossy metal which is favorable for adherence and survival of HELA cells. When the angle of incidence is closer to the critical angle, the incident light wave propagates along the surface causes the molecules of the metal to resonate. Thus, the incident light is absorbed completely, and the CCD camera captures a dark plane. However, in the regions where there are cells adhered to the metal, there is a change in refractive index which shifts the critical angle higher. In these regions, light reflects from the surface allowing us to image the signal with high contrast.

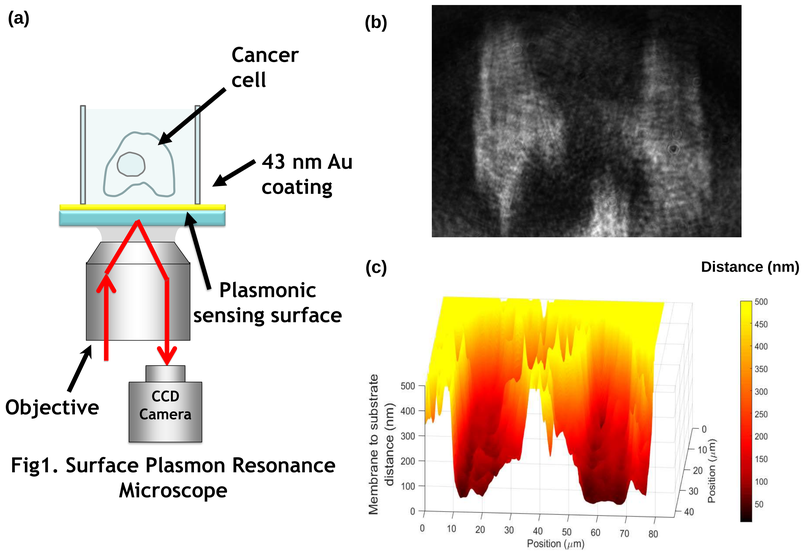

Fig1. Surface Plasmon Resonance Microscope

Figure 1. Detection principle and the plasomonic images cancer cells. (**a**) Schematic illustration of objective based plasmonic imaging microscope and the principle to measure the membrane fluctuations. (**b**) Image captured by a CCD camera displaying two cancer cells. (**c**) Three dimensional membrane map of image captured.

Results

Quantify small movement of localized membrane fluctuations:

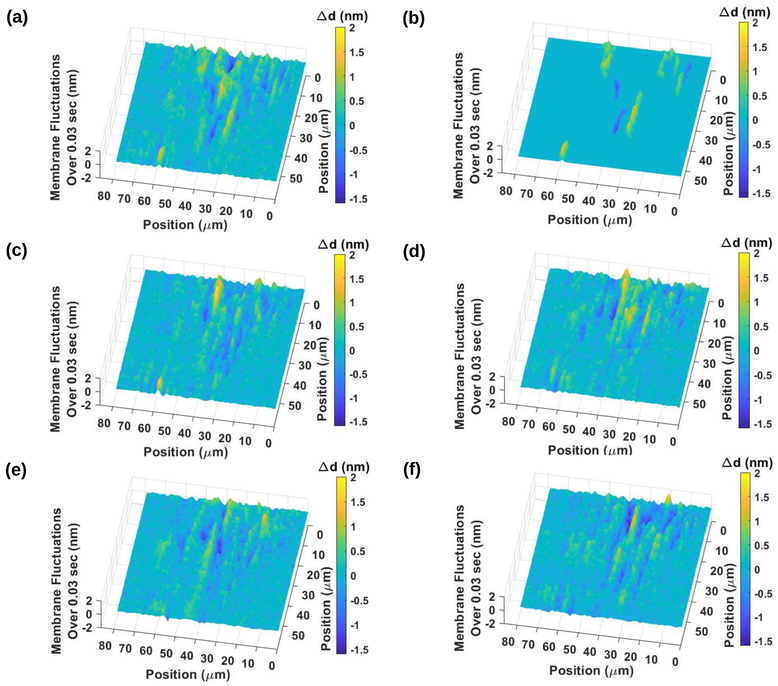

Figure 2. Observe the localized cell membrane vibrations. (**a**) Localized cell membrane fluctuations in a particular time frame, (**b**) Detect membrane fluctuations after applying morphological image processing, (**c-f**) Localized cell membrane vibrations at different time frames.

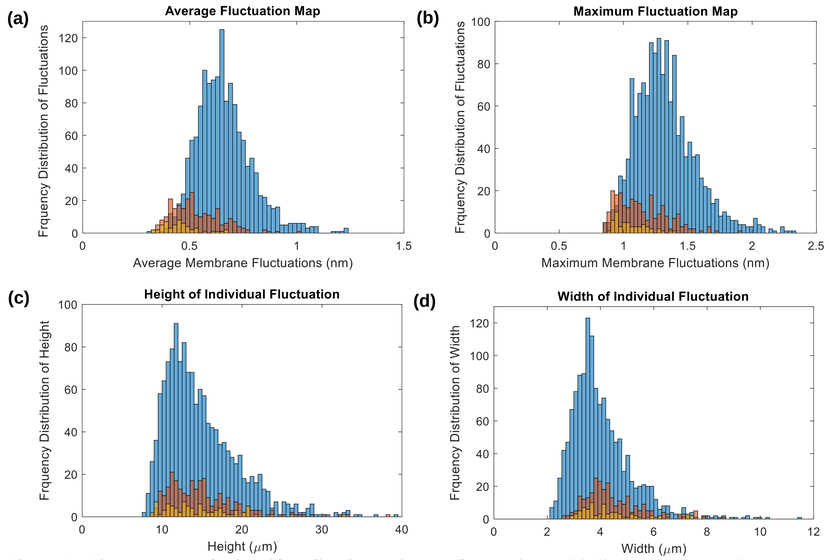

Figure 3. Histogram analysis of localized membrane fluctuations. (**a**) Fluctuation map of average membrane fluctuations, (**b**) Fluctuation map of maximum membrane fluctuations, (**c**) Height of individual fluctuation laterally and (**d**) Width of individual fluctuation laterally.

Long Duration Membrane Movement and Temporal Analysis

The cell membrane when observed for a longer duration (10 min) shows a collective membrane movement as shown below:

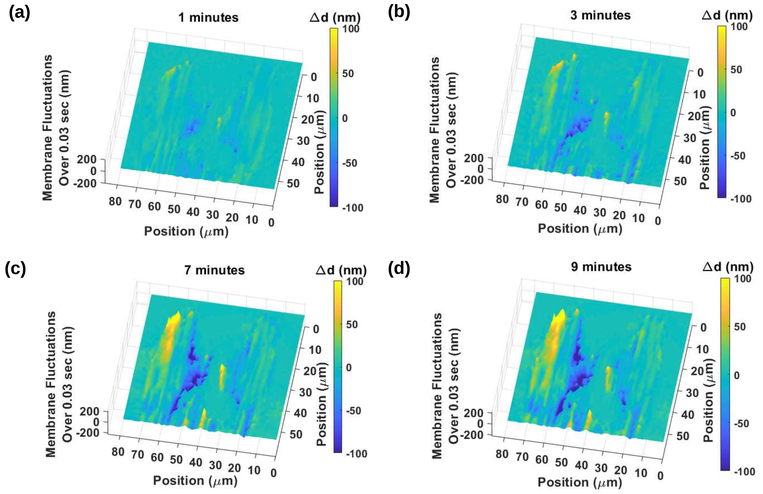

Figure 4. Collective membrane movement over a long duration of time. (**a**) After 0 minutes, (**b**) After 3 minutes, (**c**) After 7 minutes and (**d**) After 9 minutes.

Figure 5. Time domain analysis of membrane fluctuations. (**a**) Plot localized membrane fluctuations over time for 26 fps frame acquisition rate. (**b**) Power spectral density plot of part(**a**). (**c**) Plot localized membrane fluctuations over time for 106 fps frame acquisition rate. (**b**) Power spectral density plot of part(**c**).

Conclusion

- The results show that the short duration average fluctuation of cell membrane is ~1 nm,
- The average width of these localized fluctuation is ~5nm and
- The long duration fluctuation is approximately 100 nm.

Future Research and Development

- Mechanical Properties of Single Cancer Cells:
  - Determine the viscoelastic properties of the cells.
  - Determine cell structure.
- Analyze effects of drugs:
  - Study metabolism in cancer cells.
  - Analyze the effect of biomarkers when cancer cells are exposed to different drug treatments.

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