High Resolution pH Mapping of Single A549 Cells Using Scanning Ion Conductance Microscopy

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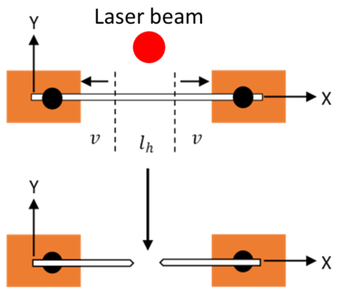
Summary

Adenocarcinoma is a common form of lung cancer, and the development of new anticancer drugs could lead to better treatment options. The anticancer drug, such as Toyocamycin, has shown effects on multiple prostate and pancreatic cancer cell-lines but has not been tested on lung carcinoma cells. Using non-invasive and label-free technique such as scanning ion conductance Microscopy (SICM), Toyocamycin was found to be effective against the A549 cell-line, inducing membrane blebbing, cell shrinkage, and apoptotic volume decrease. To further investigate its effects, poly-L-lysine (PLL) based pH sensors will be used to measure changes in the pH of the cell membrane upon drug introduction, providing insights into its mechanism of action. Additionally, the fabrication of iridium oxide pH sensors will be compared to PLL sensors to evaluate the different methodologies, the fabrication process and the potential applications of these sensors in drug studies.

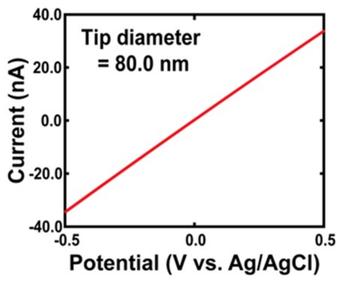
SICM as a technique for 3D live cell imaging

Fabrication of SICM imaging probe using a laser puller



Nanopipettes are fabricated from quartz capillary tube with I.D: 0.5 or 0.7 mm and O.D: 1 mm

Characterization of SICM imaging probe in 1 M KCl using cyclic voltammetry

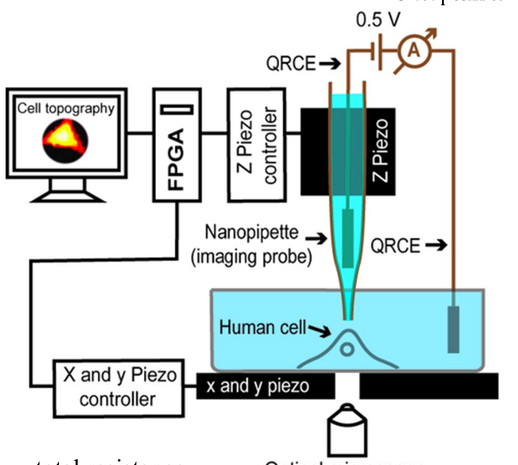


Equation used for calculating nanopipette size

$$V = IR_{\mathrm{p}} \qquad R_{\mathrm{p}} = \frac{1}{\sigma} \frac{1}{\pi r_{\mathrm{i}} \tan \alpha}$$

Advantages of SICM
- Non-invasive
- Label free
- Nanoscale resolution

Limitations
- Low throughput
- Susceptible to current drift
- Realtime studies with SICM is challenging



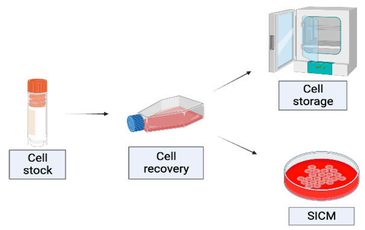
$$I(A) = \frac{V}{R_{\mathrm{p}} + R_{\mathrm{AC}} + R_{\mathrm{s}}}$$

$$R_{\mathrm{T}} = R_{\mathrm{P}} + R_{\mathrm{AC}} + R_{\mathrm{S}}$$

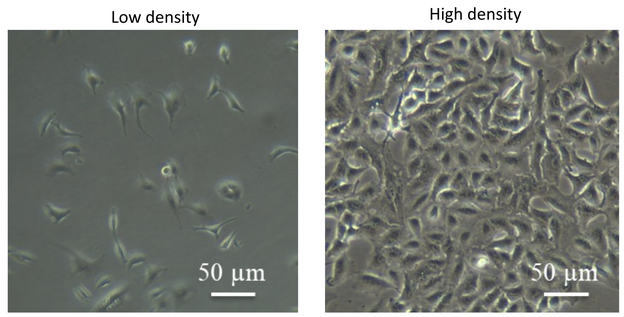
R_{T} = total resistance
R_{P} = nanopipette resistance
R_{AC} = access resistance
R_{S} = solution resistance

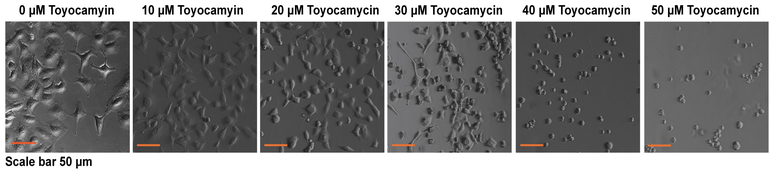
Preparation of A549 and Toyocamycin

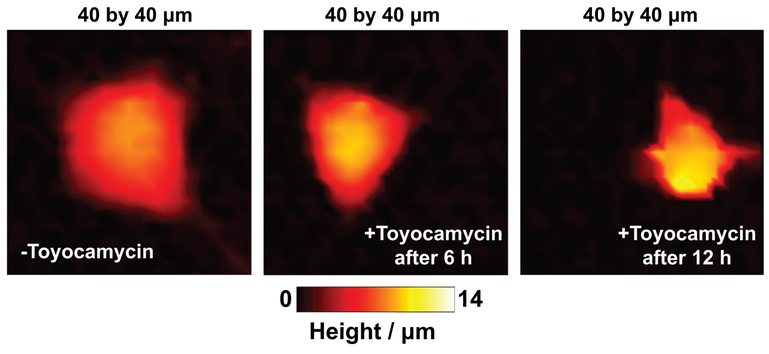
Preparation of A549 cells for SICM

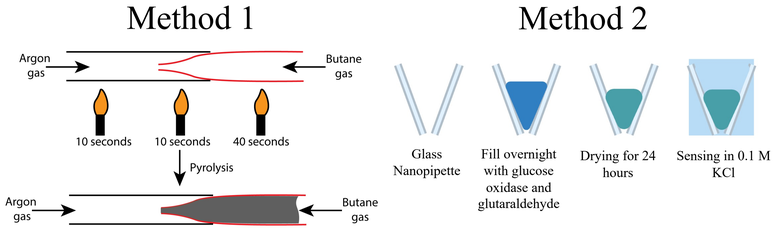


Applications of A549 cell
- Adenovirus production
- Drug studies
- CRISPR studies



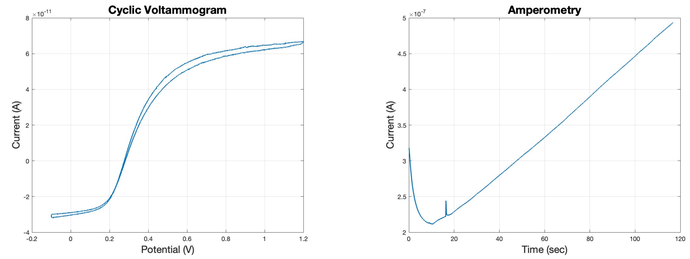






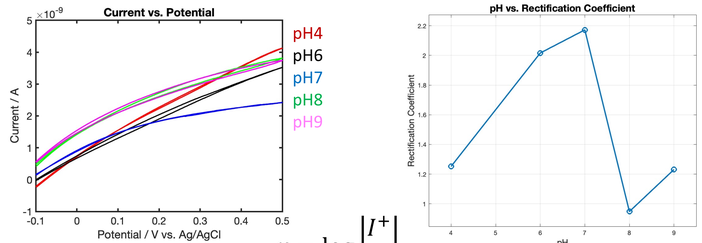
pH Sensor Calibration

Method 1



$$[\mathrm{Ir(COO)_2(OH)_2}]^{2-} \longrightarrow \mathrm{IrO_2} + 2\mathrm{CO_2} + 2\mathrm{H_2O} + 2\mathrm{e^-}$$

Method 2



$$r = \log \left| \frac{I^+}{I^-} \right|$$

Conclusion

The current method for fabricating poly-L-lysine pH sensors has demonstrated a low success rate, indicating the need for significant improvement. Developments are required for the single-barrel pH sensor to enhance the reliability and success of the sensing. To further analyze the sensor behavior and improve its consistency, a graph of pH versus its ion current rectification coefficient was included which provided no further correlation with the pH. Despite these efforts, the Iridium oxide sensors exhibited greater reliability and lower time costs, making them a more efficient option. However, further optimization is necessary for both fabrication methods to improve the overall sensor performance and ensure reproducibility in its applications.

Future Direction

The future direction for this project is to apply Scanning Ion Conductance Microscopy to the pH sensors that were fabricated and capture a 3-dimensional image of an A549 cell induced with Toyocamycin. This will allow to analyze the cell using the pH sensors to understand the effect the drug has within its cellular membrane.

References and Group Information

Zhang, Y.; Takahashi, Y.; Hong, S. P.; Liu, F.; Bednarska, J.; Goff, P. S.; Novak, P.; Shevchuk, A.; Gopal, S.; Barozzi, I.; Magnani, L.; Sakai, H.; Suguru, Y.; Fujii, T.; Erofeev, A.; Gorelkin, P.; Majouga, A.; Weiss, D. J.; Edwards, C.; Ivanov, A. P. High-Resolution Label-Free 3D Mapping of Extracellular PH of Single Living Cells. *Nature Communications* **2019**, *10* (1)

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