Quantitative Phase Imaging of Dynamic Cellular Internal Motion by Extracellular Fluid Viscosity Modulation

PRANNAY VEERABAHU[1], Thang L. Nguyen, Michael A. Teitell
[1]Department of Computational and Quantitative Biology, Department of Chemistry and Biochemistry

Abstract

Cellular motion is critical for maintaining homeostasis and physiological function, with changes in motility linked to physiological or pathological processes, such as cancer dissemination and metastasis. However, the mechanism(s) underlying motility in cancer progression need further elucidation. Studies to date indicate that cellular motion is composed of two components, an internal motion driven by movement of internal molecules against the cellular framework, and external motion through interactions with the surrounding physical environment. Motility has also been shown to influence the efficacy of cancer chemotherapy. Therefore, we hypothesize that internal motion, modulated through extracellular fluid viscosity (ECV), could contribute to drug efficacy by impeding or aiding intracellular dissemination. Using Quantitative Phase Imaging, we analyzed single-cell biomass dynamics to quantify growth heterogeneity, growth rate, and cellular proliferation under drug-stress in a controlled range of ECVs, probing a potential link between TRPV4-regulated internal motion and drug efficacy. In this study, we begin to establish internal motion as a key pharmacological variable, with contributions to drug efficacy by both the physical environment and internal motion signaling pathways.

Introduction and Hypothesis

Cellular motility plays an important role in regulating cell physiology and behavior. Literature indicates bolstered cellular motility is a driver of more aggressive cancer phenotypes, more efficient at disseminating, metastasizing, and evading anti-tumor agents. However, the underlying mechanisms linking cellular motility to cancer progression and resistance, nor the exact physical role of motility has been established.

Recent studies have established that cellular motion can be decomposed into two components: Internal and External motion. External motion is responsible for direct, polarized motion of the cell against extracellular fluid. Internal motion involves the dynamic movement, aggregation, and oscillation of cytosolic components, such as actin, against the cell's external framework. Internal motion can be modulated by extracellular fluid viscosity. Increased extracellular fluid viscosity promotes NHE1 mediated cell swelling. NHE1 serves as an upstream activator of TRPV4, a calcium ion channel. TRPV4 acts as the master regulator of the ECV-modulated internal motion pathway, localizing F-actin and RHOA to the cellular leading edge. TRPV4 mediated F-actin localization diminishes actin retrograde flow towards the cell center, reducing the magnitude and extent of internal motion observed in cell cultured in elevated extracellular fluid viscosity.

We aimed to investigate the contribution of internal cellular motion to clinically relevant cancer attributes, specifically the efficacy of doxorubicin. We hypothesized that internal motion influences drug effectiveness, whereby elevated extracellular viscosity attenuates internal motion, thereby impeding intracellular dissemination of doxorubicin and limiting its access to target sites.

We cultured MDA-MB-231 cells in-vitro in both low (0.77 cP) and high extracellular (8 cP) viscosity conditions, modulating viscosity through incorporation of methylcellulose over 1 week into culture media. Afterwards, performed cytometry (n=4 technical replicates) on MDA-MB-231 cells under 0.1 uM doxorubicin at both low and high EFV, recording cellular growth at t = 24 hour intervals over 6 days.

Then, we used Quantitative Phase Imaging (QPI) to quantify single-cell biomass distribution and associated spatial and temporal changes over 2 days. QPI uses interferometry to measure the phase shift of light as it passes through transparent cells. The velocity of light diminishes as light through dry cell mass, resulting in phase shifts. QPI quantifies a light phase shift for all elements within the cell. The integrated phase shift across the entire area of the cell is proportional to the mass within this region

In this project, we used QPI, containing both mass quantification and imaging capabilities, to quantify proliferation rate, single-cell growth rate, and their respective heterogeneity across a sample. We use these parameters to quantify drug efficacy. Afterwards, e repeated these experiments on a shTRPV4 knockdown system in MDA-MB-231 to establish the origin of ECV-modulated reduced drug efficacy as externally viscosity-related, or biologically-related internal motion.

Methodology

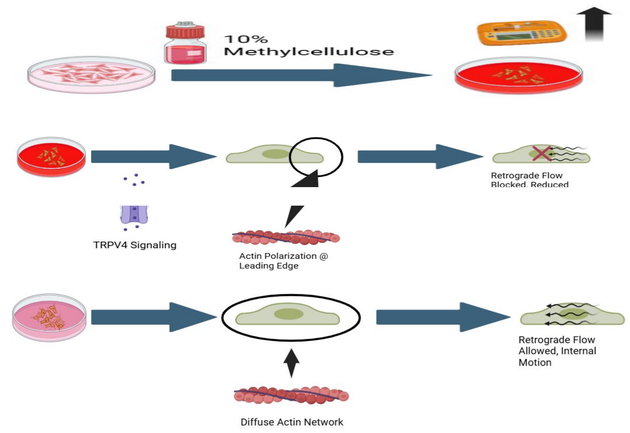
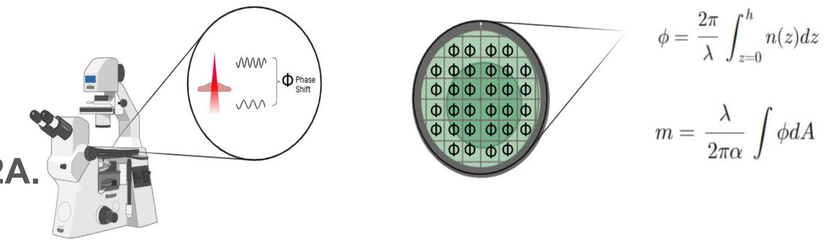


Figure 1: Modulation of Extracellular Fluid Viscosity via Methylcellulose limits internal motion through F-actin polarization at leading edge

2A.



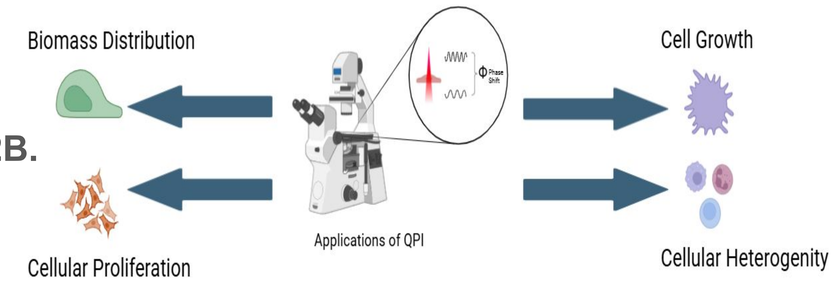


Figure 2: Introduction to Quantitative Phase Imaging. A) The phase shift of light is summated and used to compute the total dry-mass within an enclosed cellular region. B) QPI analysis is useful for measuring attributes such as growth, mass distribution, and cellular proliferation, while parsing single-cell heterogeneity among these metrics.

3A.

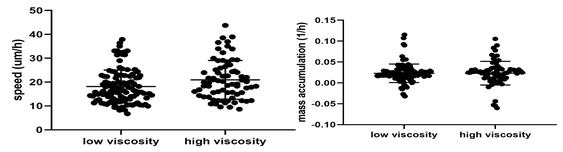
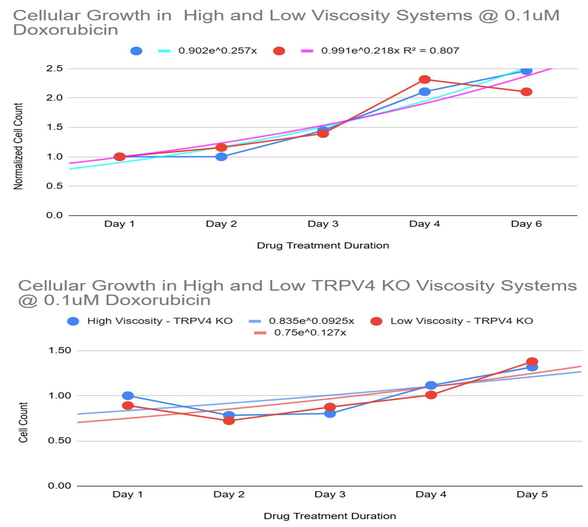


Figure 3: Verification of QPI Analysis & Preliminary Cytometry. 3A) QPI verification of successful attenuation of internal motion via extracellular fluid viscosity modulation, with increased speed (external motion) found in high EFV systems relative to comparable low EFC systems. 3B) High EFV systems demonstrate reduced doxorubicin efficacy relative to low EFV systems. This phenomena was isolated to biological internal motion signaling, with comparable drug efficacy found in both high and low EFV systems with TRPV4 Knockdown.

3B.



General Growth Formula: $F(X) = e^{cx}$

C_{lv} ~= 0.257;
$C_{lv\text{-}TRPV4\ KO}$ ~= 0.0925;
C_{hv} ~= 0.218;
$C_{hv\text{-}TRPV4\ KO}$ ~= 0.127

4.

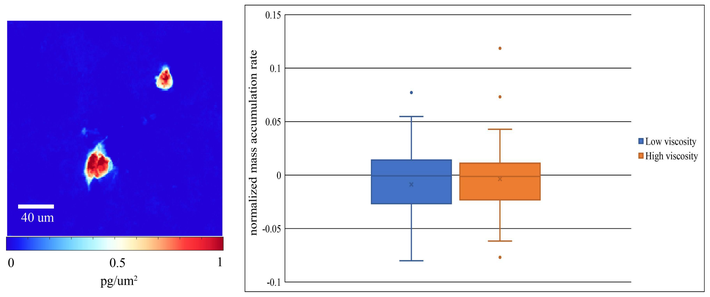


Figure 4: QPI Analysis of single-cell growth rate for high and low ECF systems. The results confirm the prior cytometry results: A greater mean growth rate (lower doxorubicin efficacy) in MDA-MB-231 cells cultured in high ECF viscosity relative to normal (low ECF) viscosity culture under doxorubicin stress.

Discussion

We first confirm QPI as an accurate and precise method of analyzing the downstream physiological effects of cellular internal motion, such as doxorubicin efficacy, through orthogonal comparison with cytometry. Moving further, we find that MDA-MB-231 cells cultured in an elevated extracellular fluid viscosity mitigate doxorubicin efficacy, verified by both cytometry and QPI. We also establish preliminary results which suggest that reduced drug efficacy in high ECF cultured cells is attenuated by TRPV4 knockdown – suggesting a link between the internal motion, regulated by the TRPV4-dependent signaling pathway, and reduced drug efficacy. However, future steps are required, namely: QPI analysis of TRPV4 KO system in high and low ECF, exploration of growth heterogeneity between low and high ECF across a spectrum of drug concentrations, verification of statistical significance, and observing if these effects persist across chemotherapy with alternative cytotoxic mechanisms.

Acknowledgments + References

I would like to thank my mentors, Dr. Thang Nguyen and Professor Michael Teitell, for their continued support, guidance, and assistance. I would also like to thank BIG Summer Program for their generous financial support, mentorship, and educational opportunities

1. **Bera, K., Kiepas, A., Godet, I. *et al.* Extracellular fluid viscosity enhances cell migration and cancer dissemination. *Nature* 611, 365–373 (2022). https://doi.org/10.1038/s41586-022-05394-6**
2. **Nguyen TL, Pradeep S, Judson-Torres RL, Reed J, Teitell MA, Zangle TA. Quantitative Phase Imaging: Recent Advances and Expanding Potential in Biomedicine. ACS Nano. 2022 Aug 23;16(8):11516-11544. doi: 10.1021/acsnano.1c11507. Epub 2022 Aug 2. PMID:**