

ROUTING AND ACTION

MEMORANDUM

ROUTING

TO:(1) Humans in Complex Systems Branch (HCxS) (Ivanisevic, Albena)

Report is available for review

(2) Proposal Files Report No.:

Proposal Number: 74808-HC.9

DESCRIPTION OF MATERIAL

CONTRACT OR GRANT NUMBER: W911NF-19-1-0306

INSTITUTION: Duke University

PRINCIPAL INVESTIGATOR: Adam P. Wax

TYPE REPORT: Final Report

DATE RECEIVED: 4/18/23 2:38PM

PERIOD COVERED: 6/12/19 12:00AM through 12/11/22 12:00AM

TITLE: Final Report: Analysis of mechanical induction of bioelectric activity in cells

ACTION TAKEN BY DIVISION

(x) Report has been reviewed for technical sufficiency and IS IS NOT satisfactory.

(x) Based on my technical review, I have identified no OPSEC or Technology Protection concerns that need to be addressed regarding this report.

(x) Performance of the research effort was accomplished in a satisfactory manner and all other technical requirements have been fulfilled.

(x) Based upon my knowledge of the research project, I agree with the patent information disclosed.

Approved by SSL\ALBENA.IVANISEVIC2 on 4/19/23 9:29AM

ARO FORM 36-E

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1. REPORT DATE (DD-MM-YYYY) 18-04-2023	2. REPORT TYPE Final Report	3. DATES COVERED (From - To) 12-Jun-2019 - 11-Dec-2022
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4. TITLE AND SUBTITLE Final Report: Analysis of mechanical induction of bioelectric activity in cells	5a. CONTRACT NUMBER W911NF-19-1-0306
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER 611102

6. AUTHORS	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES Duke University C/O Office of Research Support 2200 W. Main St., Ste. 200 Durham, NC 27705 -4677	8. PERFORMING ORGANIZATION REPORT NUMBER
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9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211	10. SPONSOR/MONITOR'S ACRONYM(S) ARO
	11. SPONSOR/MONITOR'S REPORT NUMBER(S) 74808-HC.9

12. DISTRIBUTION AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.
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13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Adam P. Wax
a. REPORT UU	b. ABSTRACT UU	c. THIS PAGE UU			19b. TELEPHONE NUMBER 919-660-5143

RPPR Final Report

as of 19-Apr-2023

Agency Code: 21XD

Proposal Number: 74808HC

Agreement Number: W911NF-19-1-0306

INVESTIGATOR(S):

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EIN: 560532129

Report Date: 11-Mar-2023

Date Received: 18-Apr-2023

Final Report for Period Beginning 12-Jun-2019 and Ending 11-Dec-2022

Title: Analysis of mechanical induction of bioelectric activity in cells

Begin Performance Period: 12-Jun-2019

End Performance Period: 11-Dec-2022

Report Term: 0-Other

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Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 2

STEM Participants: 2

Major Goals: This project aims to use a combined imaging platform to examine how external stimuli are transduced by cellular structures to produce changes in internal electrical state via modulation of ion channel transmission. In previous work, the nanoscale sensitivity of quantitative phase imaging (QPI) has been used to reveal mechanical properties of cells. In this modality, an external shear stress is applied and sub-cellular changes in mass distribution are observed with QPI. A significant advantage of this assay is that the cells are not displaced during measurement. The platform was extended by incorporating FRET (Förster resonance energy transfer)-based sensors into the QPI platform. FRET technology reports the nanoscale separation of donor and acceptor molecules. In this approach, FRET is used to report local cell conditions such as the tension experienced by loadbearing proteins or the concentration of calcium ions. The combination of FRET-based sensors with QPI enables application of the approach to study dynamic loading of cells. Traditionally, such dynamic loading has required indirect measures and complex devices but with our platform, we can directly visualize cell stresses without mechanical displacement.

To meet the goal of identifying how external stimuli are transduced by cellular structures to produce changes in internal electrical state via modulation of ion channel transmission, this project has adopted the following specific tasks: Task 1) Use measurements of FRET-based sensors and QPM to characterize signal transduction in cells under environmental challenges in ion concentration. In this task, we will develop methodology to analyze the mechanical signal transduction pathways which produce a change in cells' internal state. Cells will be engineered to express FRET-based voltage and tension sensors. As a test system, cells are exposed to heavy metal ions, which are known to both block certain ion channels and modulate cell mechanical properties, to induce changes in cell behavior. Task 2) Use measurements of FRET-based sensors and QPM to characterize signal transduction in cells under environmental challenges in mechanical load. In this aim, cell behavior is examined in response to atypical mechanical loading such as that found as metastatic cells migrate through narrow openings. The integrated optical platform will be applied to image cells as they move through a small capillary, using FRET based sensors to examine the interaction of ion concentration, alteration in sub-cellular structures, and loading of mechanosensitive proteins.

Accomplishments: 1) Major Activities

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as of 19-Apr-2023

The timeline for this project calls for work on Task 2 in Year 3. This work aligns with the final project milestones: a) using QPM to evaluate mechanical properties through disorder measurements, b) Correlating changes in mass distribution with FRET measurements of ion concentrations.

a) In this past funding period, we conducted studies of A431 cells using the combined QPI-FRET system. The measurements produced disorder strength measurements that were substantially different from previous results. We investigated this trend and realized that the disorder strength measurements were resolution dependent. We conducted experiments that examined disorder strength across resolution scales as well as digital investigations which artificially degraded the resolution. The analysis required development of a new theoretical framework that ultimately reconciled the measurements and linked the scaling trend with disorder strength.

b) in this funding period, we conducted analysis of our data set on hypo-osmotically challenged A431 cells in the presence of a FRET calcium sensor. We developed a novel framework that uses the QPI data to segment the cell by region and used this to analyze mass transport as well as the evolution of the FRET signal.

2) Specific Objectives: The objective of the project during this period is to apply our integrated optical approach to determine how changes in ion channel activity affect cell mechanical properties. The ability to monitor the time course of these intermediate steps and compare with onset of ionic stimulus and change of cell state provides unique knowledge of the dynamics of these processes in future project periods.

3) Significant results: a) We completed a study that analyzed QPM measurements of disorder strength across multiple resolution scales. This enabled measurement of the fractal dimension of mass distribution within cells, tying together two key metrics for assessing cell organization. We examined multiple cell lines with this approach and showed that each metric was able to distinguish cell phenotype and that transformed cell lines showed comparable trends for both parameters.

b) We applied the developed, combined optical system to examine intracellular transport of calcium ions using FRET and QPI. The new analysis framework enables discrimination of cell response by localized region and revealed distinct behavior for each. The analysis shows cell flattening and oscillatory mass transport in response to the stimulus.

Training Opportunities: Training Opportunities: During the past funding period, PhD Student Steven Parker and Undergraduate Albert Rancu from the Wax lab. Duke University does not use IDPs for managing trainees, instead, graduate student development is tracked using committee meetings and postdoctoral scholar development is tracked using mentor meetings.

Unfortunately, the project again saw a significant change of personnel in January 2022. Steven Parker elected to discontinue his studies during his 2nd year. This is believed to be a continuing effect from the pandemic. Steven had training opportunities including presenting an abstract on his work at the BMES conference held in October 2021 in Orlando FL. This work was selected for an oral presentation.

The work was completed with the assistance of Dr. Alexandra Munoz, a research scientist, Mr. Robert Highland III an MS student and Mr. Albert Rancu, an undergraduate student.

Robert completed analysis of the data with the assistance of Dr. Munoz and Albert. He completed his MS degree in Dec 2022 and will continue in our PhD program in Fall 2023. Albert focused on the disorder strength analysis which led to a first author manuscript which has been published in Biophysical Journal. He will continue his studies at Yale University School of Medicine.

Results Dissemination: Nothing to Report

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

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as of 19-Apr-2023

PARTICIPANTS:

Participant Type: PD/PI

Participant: Adam Wax

Person Months Worked: 1.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Co PD/PI

Participant: Brenton Hoffman

Person Months Worked: 1.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Graduate Student (research assistant)

Participant: Steven Parker

Person Months Worked: 6.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Undergraduate Student

Participant: Albert Rancu

Person Months Worked: 7.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Staff Scientist (doctoral level)

Participant: Alexandra Munoz

Person Months Worked: 5.00

Project Contribution:

National Academy Member: N

Funding Support:

ARTICLES:

RPPR Final Report as of 19-Apr-2023

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published

Journal: Cells

Publication Identifier Type: DOI

Publication Identifier: 10.3390/cells10092455

Volume: 10

Issue: 9

First Page #: 2455

Date Submitted: 8/30/22 12:00AM

Date Published: 9/1/21 4:00AM

Publication Location:

Article Title: Single Cell Analysis of Stored Red Blood Cells Using Ultra-High Throughput Holographic Cytometry

Authors: Han-Sang Park, Hillel Price, Silvia Ceballos, Jen-Tsan Chi, Adam Wax

Keywords: high throughput cell screening; quantitative phase imaging; red blood cell storage

Abstract: Holographic cytometry is introduced as an ultra-high throughput implementation of quantitative phase imaging of single cells flowing through parallel microfluidic channels. Here, the approach was applied for characterizing the morphology of individual red blood cells during storage under regular blood bank conditions. Samples from five blood donors were examined, over 100,000 cells examined for each, at three time points. The approach allows high-throughput phase imaging of a large number of cells, greatly extending our ability to study cellular phenotypes using individual cell images. Holographic cytology images can provide measurements of multiple physical traits of the cells, including optical volume and area, which are observed to consistently change over the storage time. In addition, the large volume of cell imaging data can serve as training data for machine-learning algorithms. For the study here, logistic regression was used to classify the cells according to the storage time

Distribution Statement: 1-Approved for public release; distribution is unlimited.

Acknowledged Federal Support: Y

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published

Journal: Biophysical Journal

Publication Identifier Type: DOI

Publication Identifier: 10.1016/j.bpj.2023.03.005

Volume:

Issue:

First Page #:

Date Submitted: 3/25/23 12:00AM

Date Published: 3/1/23 5:00AM

Publication Location:

Article Title: Multiscale optical phase fluctuations link disorder strength and fractal dimension of cell structure

Authors: Albert Rancu, Cindy X. Chen, Hillel Price, Adam Wax

Keywords: Fractal dimension, disorder strength

Abstract: Optical methods for examining cellular structure based on endogenous contrast rely on analysis of refractive index changes to discriminate cell phenotype. These changes can be visualized using techniques such as phase contrast microscopy, detected by light scattering, or analyzed numerically using quantitative phase imaging. The statistical variations of refractive index at the nanoscale can be quantified using disorder strength, a metric seen to increase with neoplastic change. In contrast, the spatial organization of these variations is typically characterized using a fractal dimension, which is also seen to increase with cancer progression. Here, we seek to link these two measurements using multiscale measurements of optical phase to calculate disorder strength and in turn to determine the fractal dimension of the structures. First, quantitative phase images are analyzed to show that the disorder strength metric changes with resolution. The trend of disorder strength with length sca

Distribution Statement: 1-Approved for public release; distribution is unlimited.

Acknowledged Federal Support: Y

CONFERENCE PAPERS:

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published

Conference Name: Novel Techniques in Microscopy 2021

Date Received: 28-Aug-2021

Conference Date: 12-Apr-2021

Date Published:

Conference Location: Virtual

Paper Title: Combined Quantitative Phase Microscopy and Förster Resonance Energy Transfer for Analyzing Cell Ion Dynamics

Authors: Steven M. Parker, Hillel Price, Meghan Reynolds, Siyan He, Brenton D. Hoffman, and Adam Wax

Acknowledged Federal Support: Y

RPPR Final Report
as of 19-Apr-2023

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: Label-free Biomedical Imaging and Sensing (LBIS) 2022
Date Received: 30-Aug-2022 Conference Date: 22-Jan-2022 Date Published:
Conference Location: San Francisco, United States
Paper Title: Cell disorder strength and cellular stiffness analysis across quantitative phase imaging systems
Authors: Cindy X. Chen, Albert Rancu, Steven M. Parker, Hillel Price, Adam Wax
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: Clinical and Translational Biophotonics
Date Received: 30-Aug-2022 Conference Date: 24-Apr-2022 Date Published:
Conference Location: Fort Lauderdale, Florida
Paper Title: Analysis of Disorder Strength Across Quantitative Phase Imaging Systems
Authors: Albert Rancu, Cindy X. Chen, Steven Parker, and Adam Wax
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: Microscopy Histopathology and Analytics
Date Received: 30-Aug-2022 Conference Date: 24-Apr-2022 Date Published:
Conference Location: Fort Lauderdale, Florida
Paper Title: Combined Quantitative Phase Microscopy and Förster Resonance Energy Transfer Imaging for Analyzing Endothelial Cell Shear Stress Response
Authors: Trey Highland, Steven M. Parker, Hillel Price, Meghan Reynolds, Siyan He, Brenton D. Hoffman, and Ac
Acknowledged Federal Support: **Y**

Partners

I certify that the information in the report is complete and accurate:

Signature: Adam Wax

Signature Date: 4/18/23 2:38PM

Final project report: Analysis of mechanical induction of bioelectric activity in cells

Wax lab, Hoffman lab
Department of Biomedical Engineering
Duke University

Project overview

- Project seeks to develop a platform for examining structural, mechanical, and electrical properties of cells at the nanoscale based on optical technologies.
- Study how external stimuli are transduced by cellular structures to produce changes in internal electrical state via modulation of ion channel transmission.

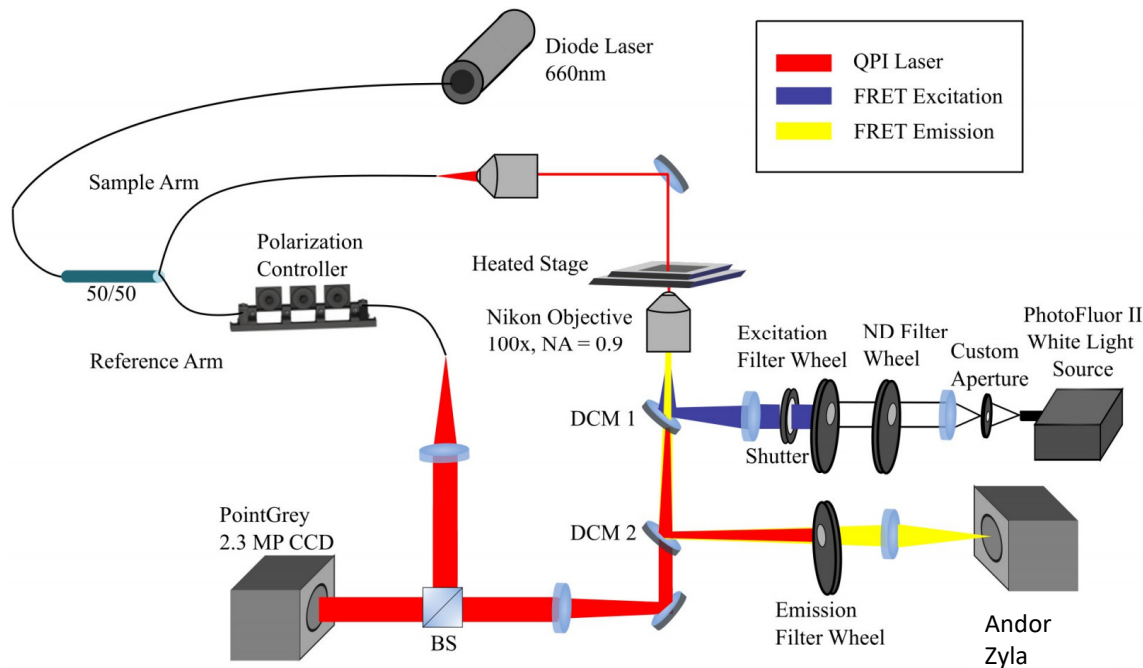
Tasks

- Task 1: Use measurements of FRET-based sensors and QPM to characterize signal transduction in cells under environmental challenges in ion concentration
- Task 2: Use measurements of FRET-based sensors and QPM to characterize signal transduction in cells under environmental challenges in mechanical load

Project Timeline and Milestones

- Year 1: Task 1: Engineer cells to express EzrTS, verify FRET signal with combined QPI/FRET system. Adapt system to enable imaging of FRET based Calcium sensor Status: Completed
- Year 2: Task 1: Conduct experiments of A431 with heavy metal exposure. Develop framework for analysis of combined QPM & FRET (tension, ion concentration) data. Task 2: Implement microfluidic platform for cell squeezing experiments. Status: In progress
- Year 3: Task 2: Conduct experiments in microfluidics that use QPM measured disorder strength to evaluate mechanical properties. Correlate changes in mass distribution with FRET measurements of tension and ion concentrations. Status: In progress

Fig 1: System Diagram



QPI-FRET system

This system is unique in that it offers high temporal throughput for QPM nanostructure measurements to monitor dynamic changes in cellular structure, such as mechanical strain via shear flow, while also providing multiple channels of fluorescence excitation and emission detection using filter wheels and a high sensitivity sCMOS camera.

Fig. 2 QPI/FRET imaging

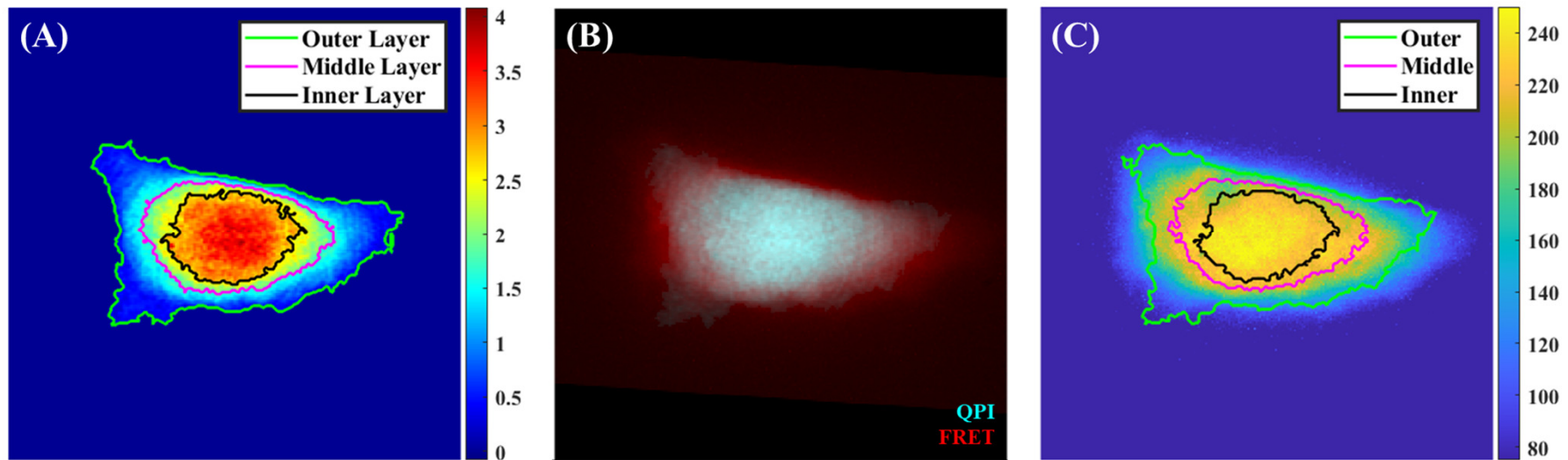
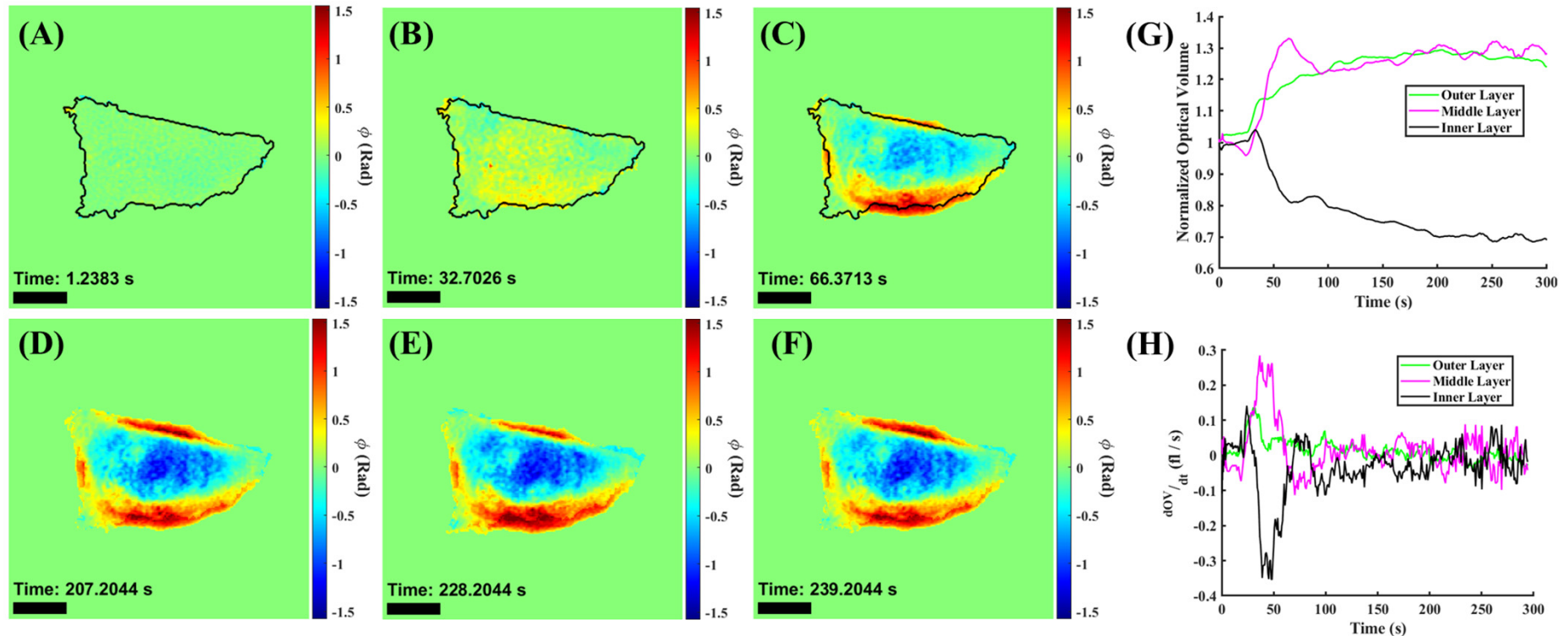


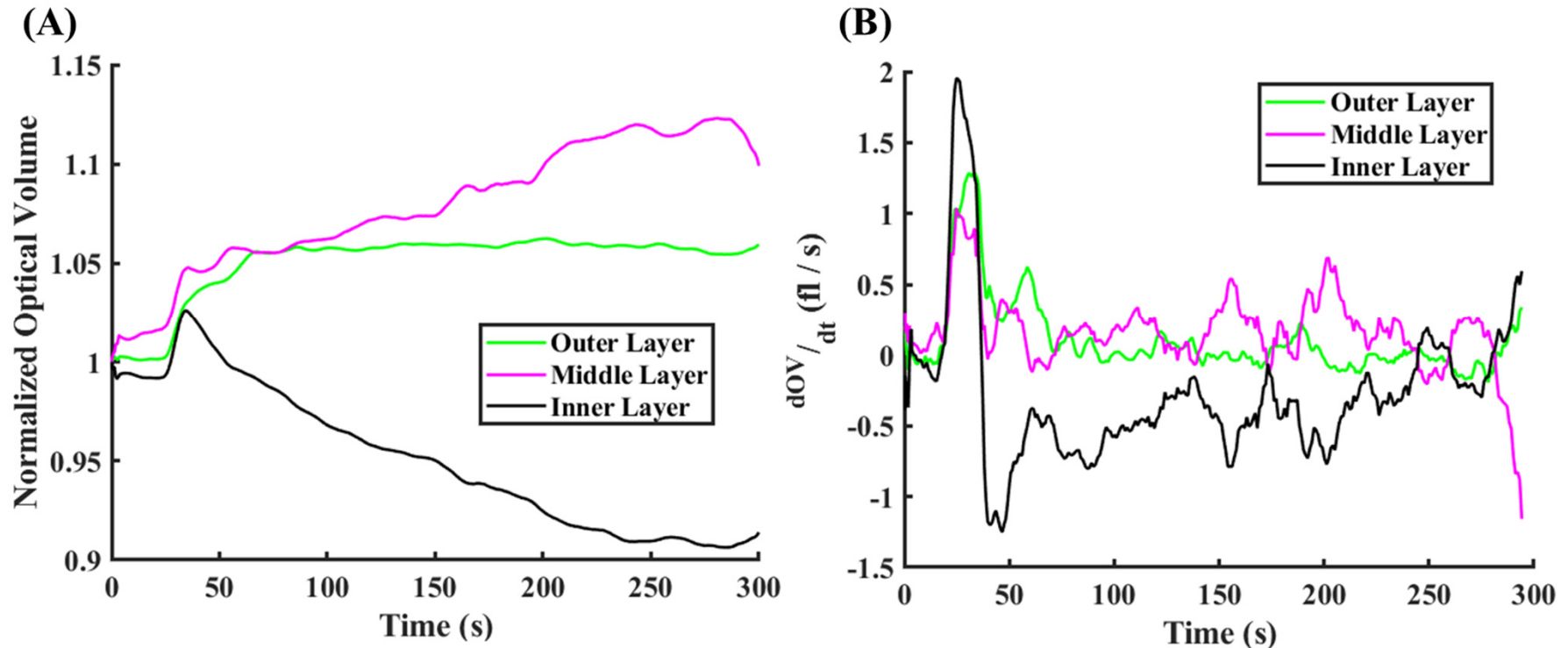
Fig 2(A) shows a QPI image in which we have used the structural data to determine an outer, middle and inner segment. Fig. 4(B) shows fused imaging of QPI (red channel) and FRET (blue channel) data. Fig 4(C) shows the analysis approach of using QPI segmented layers applied to the co-registered FRET image

Fig. 3: QPI time-lapse under osmotic challenge



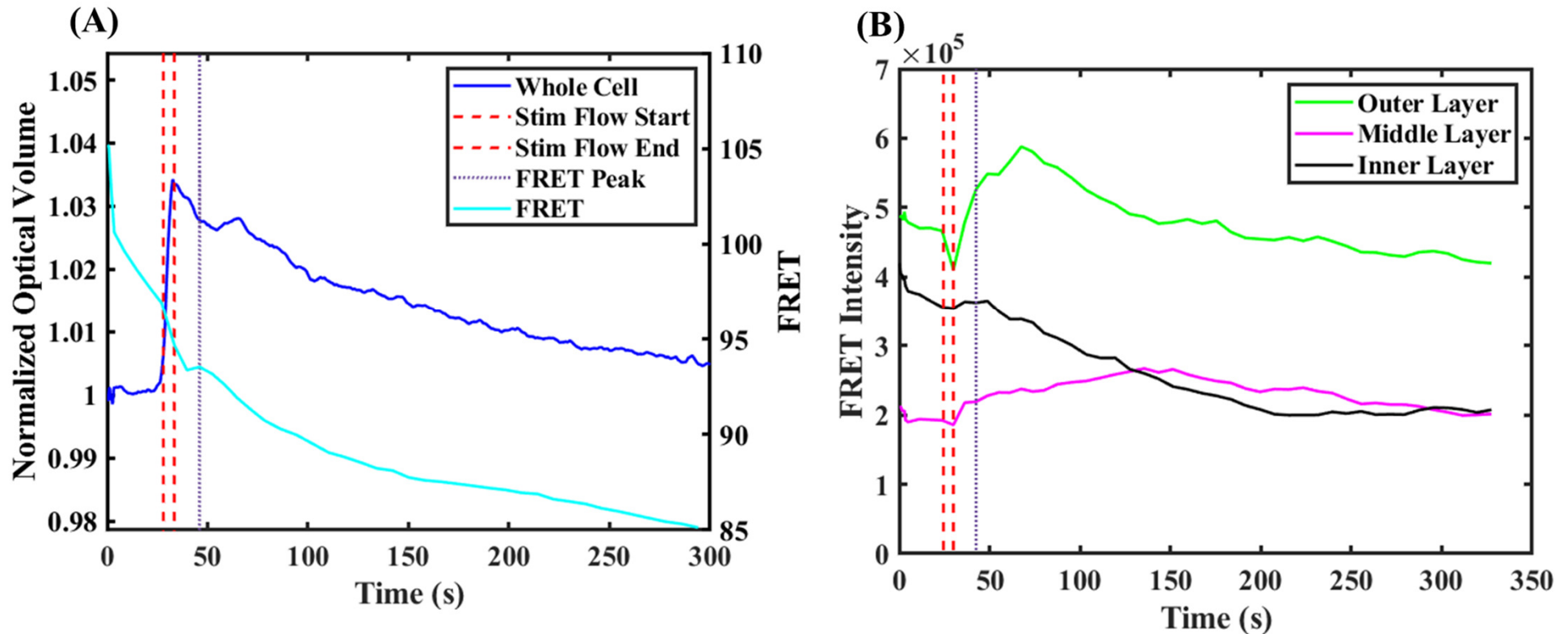
QPI data for a given cell (A)-(F), illustrating the displacement of materials as measured with QPI at various timepoints after osmotic challenge. Initially, no displacement is seen but immediately following stimulus (B, $t = 32$ s), the center of the cell shows an increase in optical volume, indicating the uptake of ions. At later times, the center of the cell is seen to decrease in mass as the material moves out to the periphery of the cell (C). At later times, the cell material appears to oscillate back and forth (D-F). The summary figures (G,H) show the changes in optical volume in each of the outer/middle/inner segments across the cell over time. The oscillatory character is evident in the middle layer (pink line).

Fig. 4 Changes in optical volume across cell population after osmotic challenge



Time course of changes in optical volume before and after osmotic stimulus. These trends are averaged over the (29) individual cells where we recorded their FRET and QPI response/ The optical volume (A) shows a few percent change in the inner layer followed by a decline while the middle layer shows an increase and then continues for several minutes. In contrast, the outer layer shows a delayed increase as seen by the offset change in slope (B, green peak). The oscillatory features at later times are noteworthy as these are still fairly clear even after taking average across populations.

Fig. 5: FRET signal across population



Trends in FRET signal across the population. The osmotic stimulus (red dashed line) causes a sharp change in optical volume (Dark Blue, left vertical axis) followed by a recovery. The FRET signal decreases steadily due to photobleaching (Light Blue). Upon removing this trend, we determine that the peak FRET signal, corresponding to the largest Ca ion concentration occurs a few seconds later. In Fig. 7 (B) the segmentation derived from QPI shows the ion flow inside the cell

Figure 6: Downsampling of QPI images

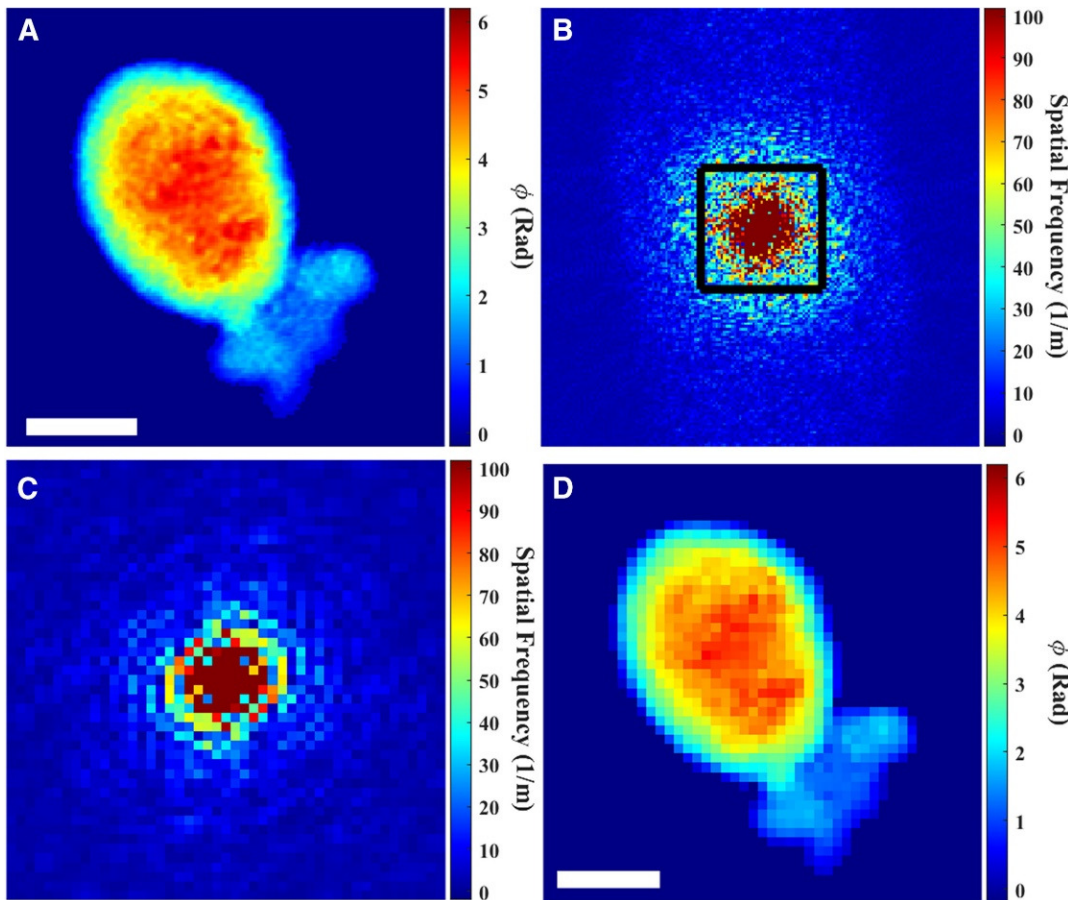


Figure 6. (A) QPI image of BT474 cell at Hi resolution. (B) 2D [Fourier transform](#) of QPI image. The black box outline represents the range of frequencies sampled for and is determined by the squared ratio of old pixel size to new pixel size multiplied by the original image size. (C) Resulting frequency space representation of QPI [image](#) [after](#) selecting from frequencies. (D) Final QPI image at Med resolution after applying a reverse Fourier transform. Scale bars, 10 μm .

Figure 7: Phase fluctuations scales with pixel size

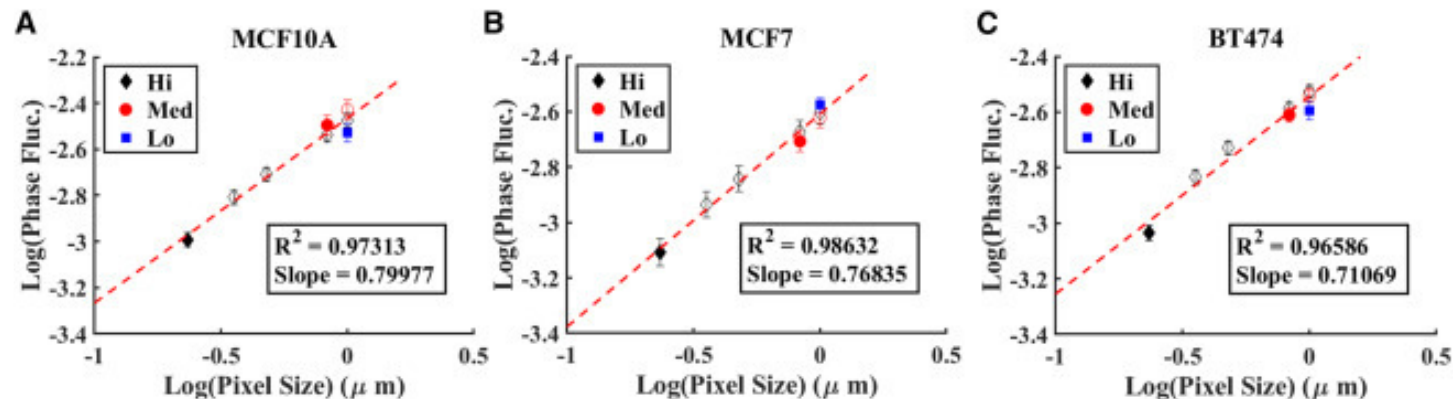
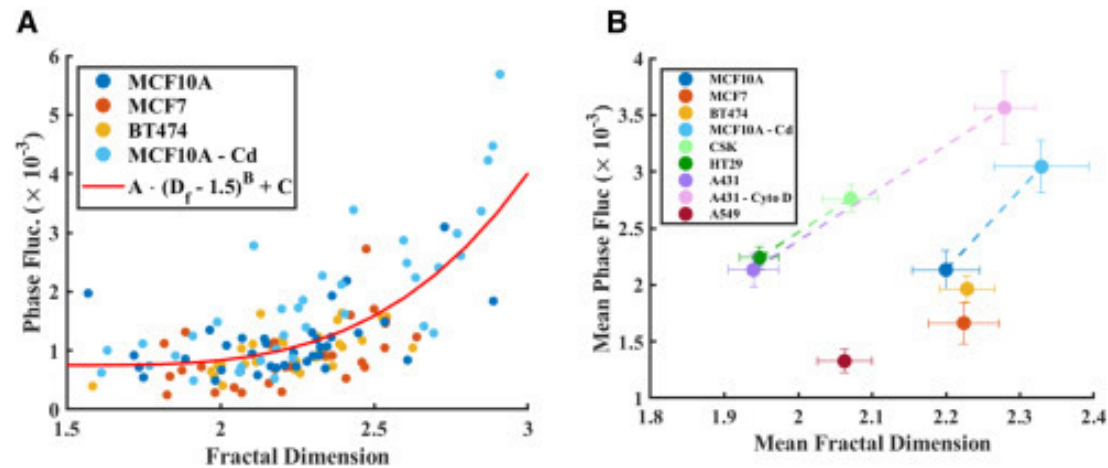


Figure 7. Phase fluctuations scales with pixel size. The symbol shape represents the QPI system resolution that the image was originally obtained with. Solid coloring represents disorder strength measurement on the native QPI image, while empty symbols (no color fill) represent disorder strength measurement on the simulated image. (A) Log-log plot of disorder strength (mean \pm SE bars) versus pixel size for MCF10A cells ($N_{\text{Hi}} = 36$, $N_{\text{Med}} = 38$, $N_{\text{Lo}} = 32$). (B) Log-log plot of disorder strength versus pixel size for MCF7 cells ($N_{\text{Hi}} = 28$, $N_{\text{Med}} = 38$, $N_{\text{Lo}} = 38$). (C) Log-log plot of disorder strength versus pixel size for BT474 cells ($N_{\text{Hi}} = 32$, $N_{\text{Med}} = 37$, $N_{\text{Lo}} = 43$).

Figure 8: D_f -phase variance analysis at single-cell and population level



(A) D_f -phase variance plot for individual MCF10A, MCF7, BT474, and MCF10A-Cd cells ($n = 133$) (mean \pm SE bars). (B) D_f -phase variance plot for all cell populations mentioned previously, as well as A549 lung cancer cells ($n = 30$).