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a. REPORT	b. ABSTRACT	c. THIS PAGE			Adam P. Wax
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RPPR Final Report
as of 19-Apr-2023

Agency Code: 21XD

Proposal Number: 74808HC **Agreement Number: W911NF-19-1-0306**
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DUNS Number: 044387793 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

Submitted By: Adam P. Wax Email: a.wax@duke.edu
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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

RPPR Final Report
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 stiumulus.

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 att he BMES conference held in October 2021 in Orlando FL. This work was selected for an oral presentation.

The work was completed with the assitance 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 assitance 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

RPPR Final Report
as of 19-Apr-2023

PARTICIPANTS:

Participant Type: PD/PI
Participant: Adam Wax
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Co PD/PI
Participant: Brenton Hoffman
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Steven Parker
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Undergraduate Student
Participant: Albert Rancu
Person Months Worked: 7.00 **Funding Support:**
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Participant Type: Staff Scientist (doctoral level)
Participant: Alexandra Munoz
Person Months Worked: 5.00 **Funding Support:**
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Publication Location:
Article Title: Shear Modulus Measurement by Quantitative Phase Imaging and Correlation with Atomic Force Microscopy
Authors: Will J.Eldridge1SilviaCeballos1TejankShah1Han SangPark1Zachary A.Steelman1StefanZauscher1Adai
Keywords: Cell biophysics, interferometry, atomic force microscopy
Abstract: Many approaches have been developed to characterize cell elasticity. Among these, atomic force microscopy (AFM) combined with modeling has been widely used to characterize cellular compliance. However, such approaches are often limited by the difficulties associated with using a specific instrument and by the complexity of analyzing the measured data. More recently, quantitative phase imaging (QPI) has been applied to characterize cellular stiffness by using an effective spring constant. This metric was further correlated to mass distribution (disorder strength) within the cell. However, these measurements are difficult to compare to AFM-derived measurements of Young's modulus. Here, we describe, to our knowledge, a new way of analyzing QPI data to directly retrieve the shear modulus. Our approach enables label-free measurement of cellular mechanical properties that can be directly compared to values obtained from other rheological methods. To demonstrate the technique, we measured she
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Article Title: Quantitative phase imaging of erythrocytes under microfluidic constriction in a high refractive index medium reveals water content changes
Authors: Han Sang Park1 , Will J. Eldridge1 , Wen-Hsuan Yang 2,3,4, Michael Crose1 , Silvia Ceballos1 , John D
Keywords: Cell biophysics, interferomtery, microfluidics
Abstract: Changes in the deformability of red blood cells can reveal a range of pathologies. For example, cells which have been stored for transfusion are known to exhibit progressively impaired deformability. Thus, this aspect of red blood cells has been characterized previously using a range of techniques. In this paper, we show a novel approach for examining the biophysical response of the cells with quantitative phase imaging. Specifically, optical volume changes are observed as the cells transit restrictive channels of a microfluidic chip in a high refractive index medium. The optical volume changes indicate an increase of cell's internal density, ostensibly due to water displacement. Here, we characterize these changes over time for red blood cells from two subjects. By storage day 29, a significant decrease in the magnitude of optical volume change in response to mechanical stress was witnessed. The exchange of water with the environment due to mechanical stress is seen to modulate with s
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Date Submitted: 8/30/22 12:00AM Date Published: 9/1/21 4:00AM
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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
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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
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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
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Authors: Cindy X. Chen, Albert Rancu, Steven M. Parker, Hillel Price, Adam Wax
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Paper Title: Analysis of Disorder Strength Across Quantitative Phase Imaging Systems
Authors: Albert Rancu, Cindy X. Chen, Steven Parker, and Adam Wax
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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

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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

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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.

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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

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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

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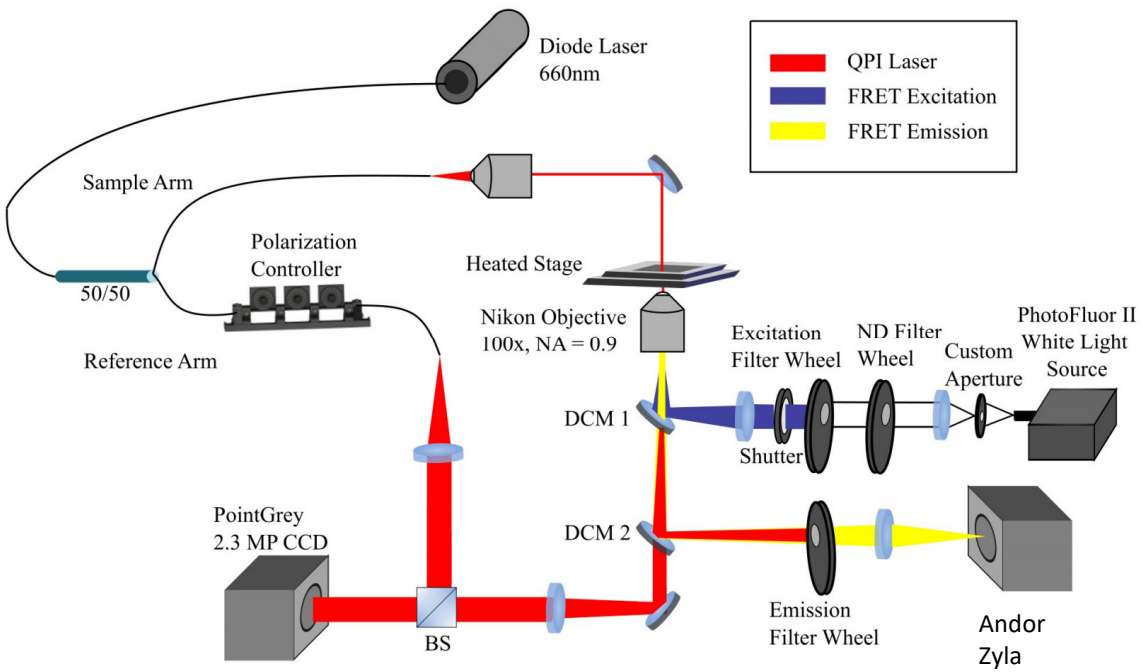
Fig 1: System Diagram



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.

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Fig. 2 QPI/FRET imaging

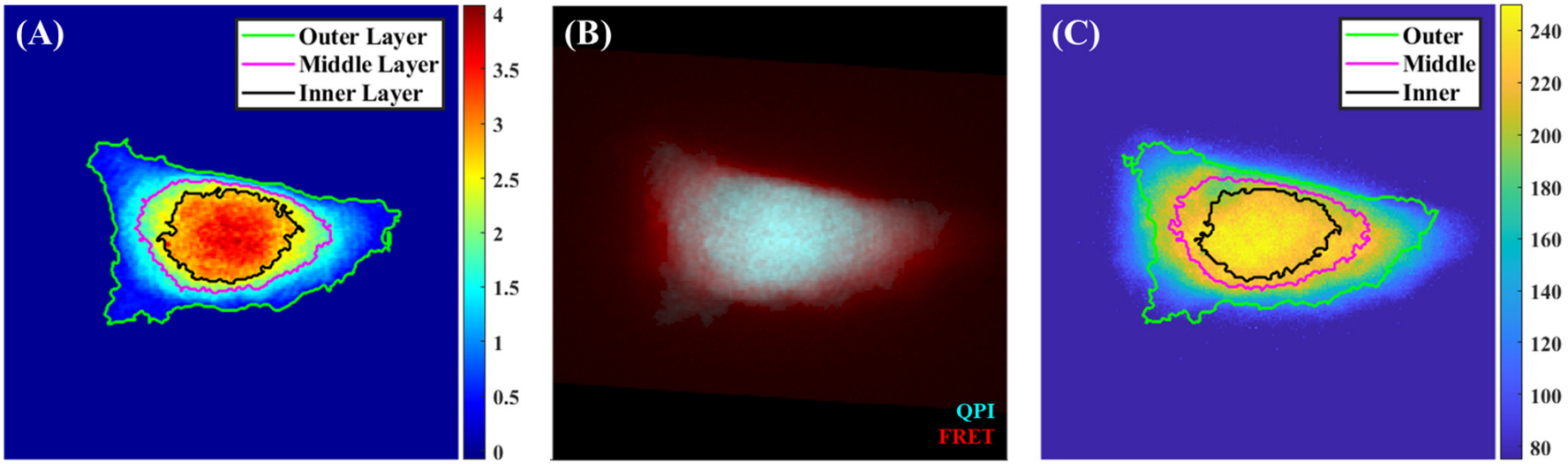

Fig 2(A) shows a QPI image in which we have used the structural data to determine an outer, middle and inner segment. Fig. 4(B) shows fused imaging of QPI (red channel) and FRET (blue channel) data. Fig 4(C) shows the analysis approach of using QPI segmented layers applied to the co-registered FRET image

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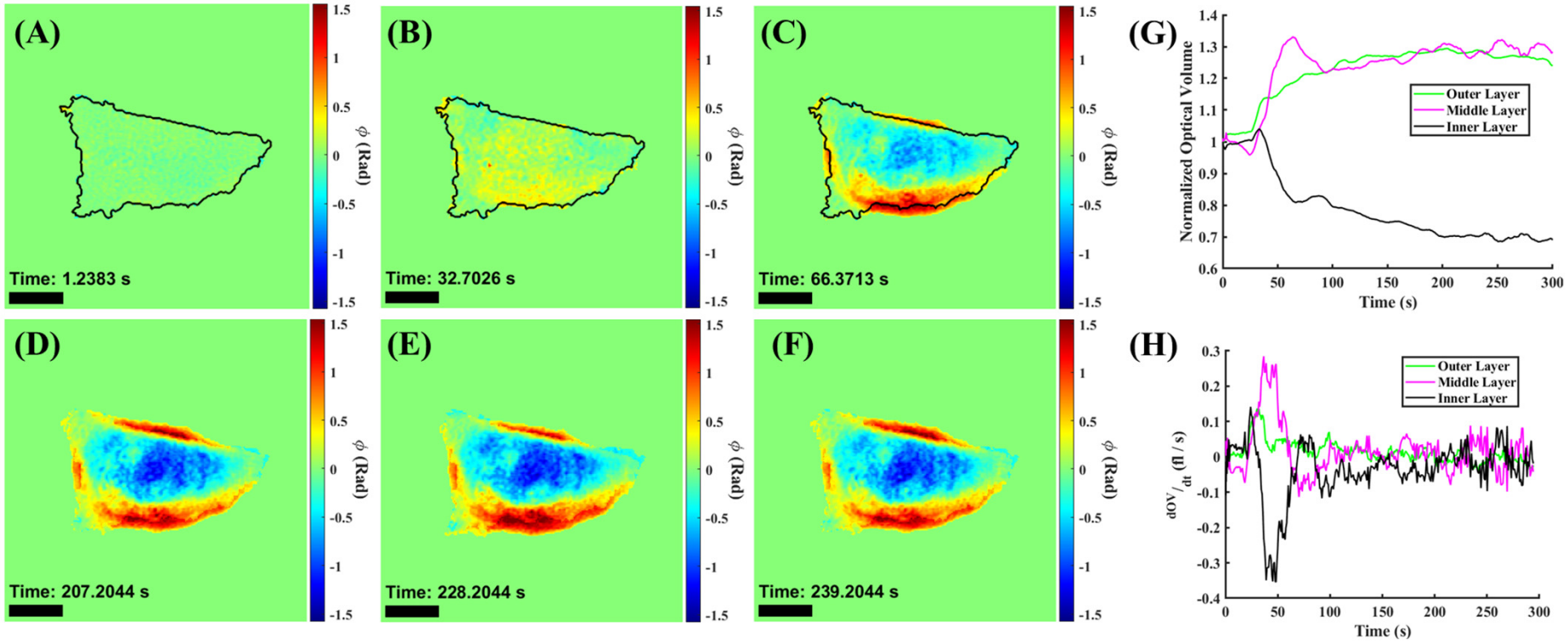
Fig. 3: QPI time-lapse under osmotic challenge



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 osciallate 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).

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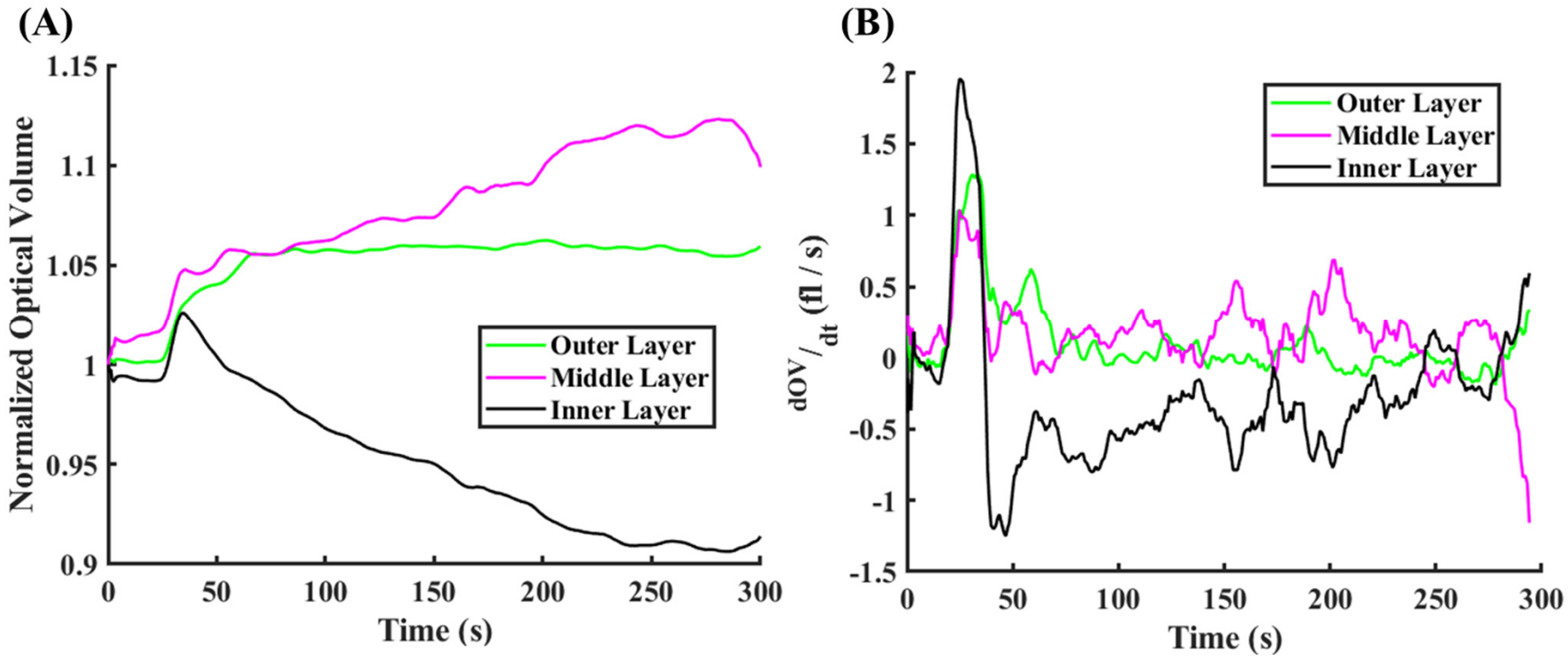
Fig. 4 Changes in optical volume across cell population after osmotic challenge



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.

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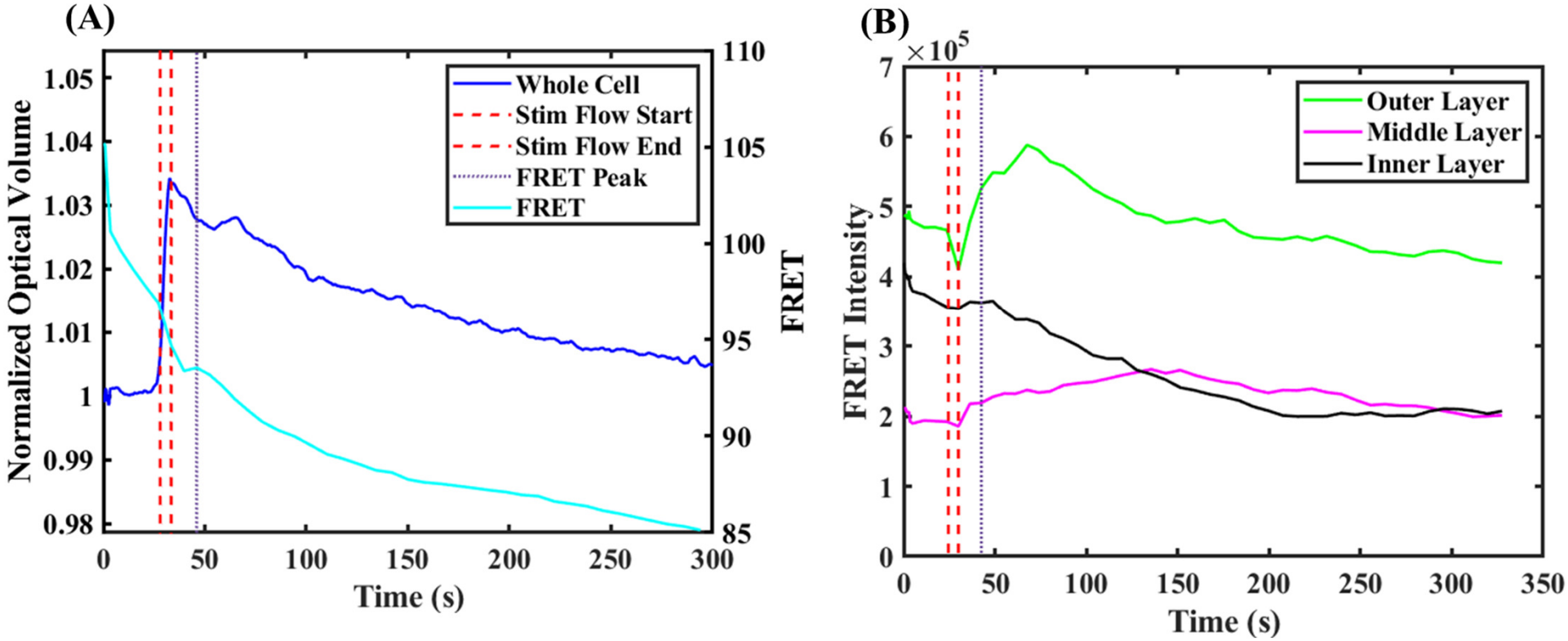
Fig. 5: FRET signal across population



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

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Figure 6: Downsampling of QPI images

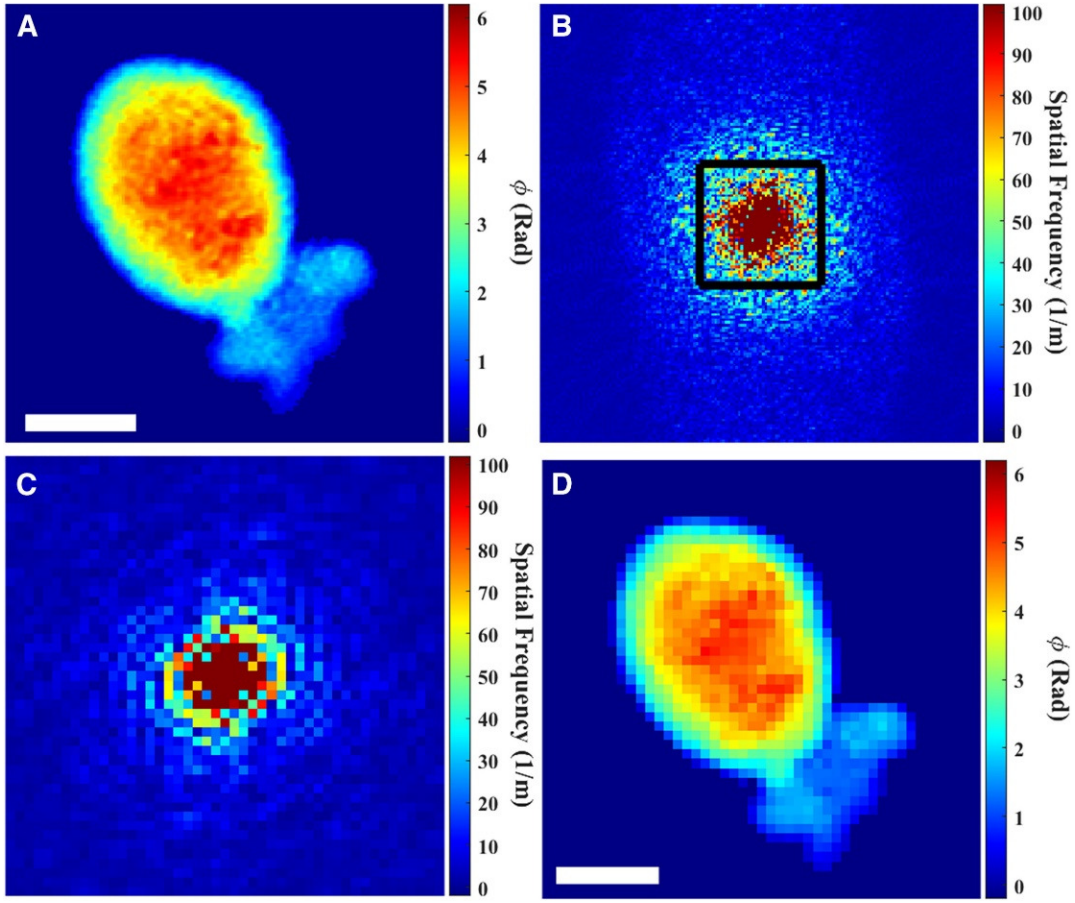

Figure 6. (*A*) QPI image of BT474 cell at Hi resolution. (*B*) 2D Fourier transform of QPI image. The black box outline represents the range of frequencies sampled for and is determined by the squared ratio of old pixel size to new pixel size multiplied by the original image size. (*C*) Resulting frequency 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.

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Figure 7: Phase fluctuations scales with pixel size

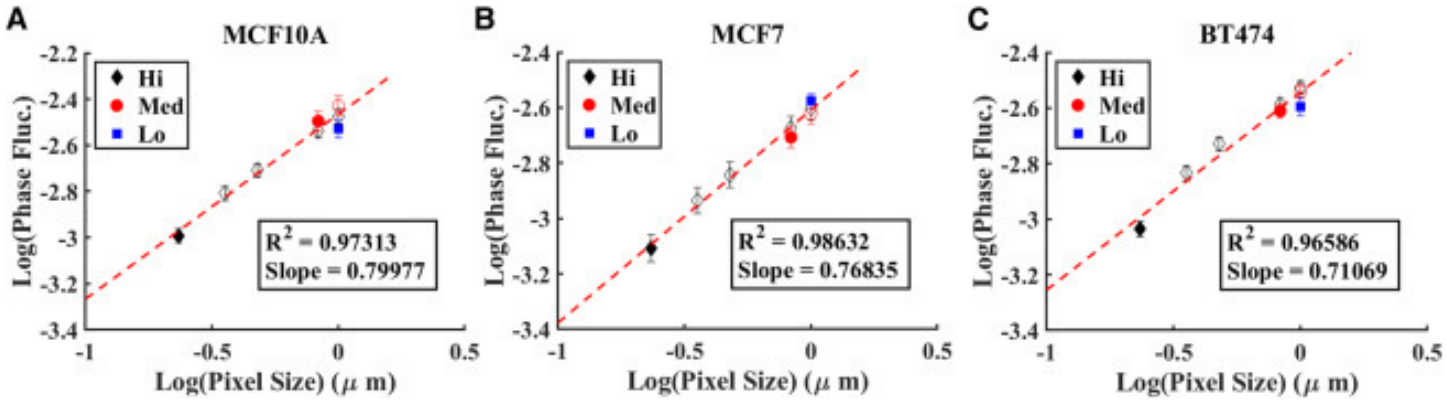

Figure 7. Phase fluctuations scales with pixel size. The symbol shape represents the QPI system resolution that the image was originally obtained with. Solid coloring represents disorder strength measurement on the native QPI image, while empty symbols (no color fill) represent disorder strength measurement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

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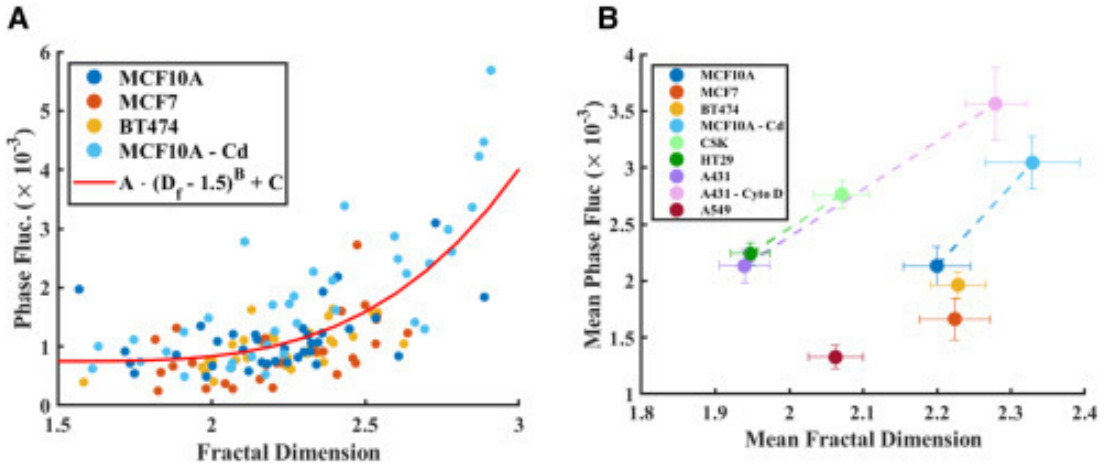
Figure 8: Df-phase variance analysis at single-cell and population level



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