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TITLE: "Quantitative Phase Microscopy for Real-Time Clinical Determination of Drug Therapy Response in Primary and Metastatic Breast Cancer"

PRINCIPAL INVESTIGATOR: Philip S Bernard

CONTRACTING ORGANIZATION: University of Utah
SALT LAKE CITY, UT

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14. ABSTRACT

There are no biomarkers that can accurately predict chemotherapy response in advanced cancer patients and less than 10% of patients with a detected targetable mutation are eligible for a clinical trial. There is a need for new diagnostic methods that can accurately stratify high-risk patients to effective, FDA-approved therapies. Our current patient-derived models for assessing tumor drug response involve expanding patient tumor cells as 3D patient derived organoids (PDO) in Matrigel or using in vivo drug sensitivity studies with patient-derived xenograft models (PDX). These experimental models typically exhibit the same phenotype and molecular alterations in vivo and ex vivo and have the same drug responses as in the patient. However, these methods require 1-8 months to obtain drug sensitivity profiles making this impractical for patient care. In this project we will develop a functional assay with the new capability to predict cancer cell response to therapy within 2 d of sample collection in terms of both population response and single-cell heterogeneity. In year 1 we have developed the technical platform for this work which will be applied in years 2 and 3. Overall, our project will provide real-time feedback to oncologists on drug sensitivity/resistance and resistant subpopulations.

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1. **INTRODUCTION:**

There are no biomarkers that can accurately predict chemotherapy response in advanced cancer patients and less than 10% of patients with a detected targetable mutation are eligible for a clinical trial. There is a need for new diagnostic methods that can accurately stratify high-risk patients to effective, FDA-approved therapies. Our current patient-derived models for assessing tumor drug response involve expanding patient tumor cells as 3D patient derived organoids (PDO) in Matrigel or using *in vivo* drug sensitivity studies with patient-derived xenograft models (PDX). These experimental models typically exhibit the same phenotype and molecular alterations *in vivo* and *ex vivo* and have the same drug responses as in the patient. However, these methods require 1-8 months to obtain drug sensitivity profiles making this impractical for patient care. In this project we will develop a functional assay with the new capability to predict cancer cell response to therapy within 2 d of sample collection in terms of both population response and single-cell heterogeneity. In year 1 we have developed the technical platform for this work which will be applied in years 2 and 3. Overall, our project will provide real-time feedback to oncologists on drug sensitivity/resistance and resistant subpopulations.

2. **KEYWORDS:**

Quantitative phase imaging, microscopy, precision medicine, breast cancer

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

The SOW lists three major tasks to be accomplished during this project:

Major Task 1: Implement QPM imaging method (Zangle)
Target completion date: November 2019
Completion percentage: 100%

Major Task 2: Benchmark testing of full 26 drug panel on 8 commercially available cell lines (Zangle/Bernard)
Target completion date: March 2021
Completion percentage: 10%

Major Task 3: Conduct initial feasibility studies to assess the ability to predict chemotherapy response with previously collected, de-identified patient samples (Zangle/Bernard)
Target completion date: March 2022
Completion percentage: 5%

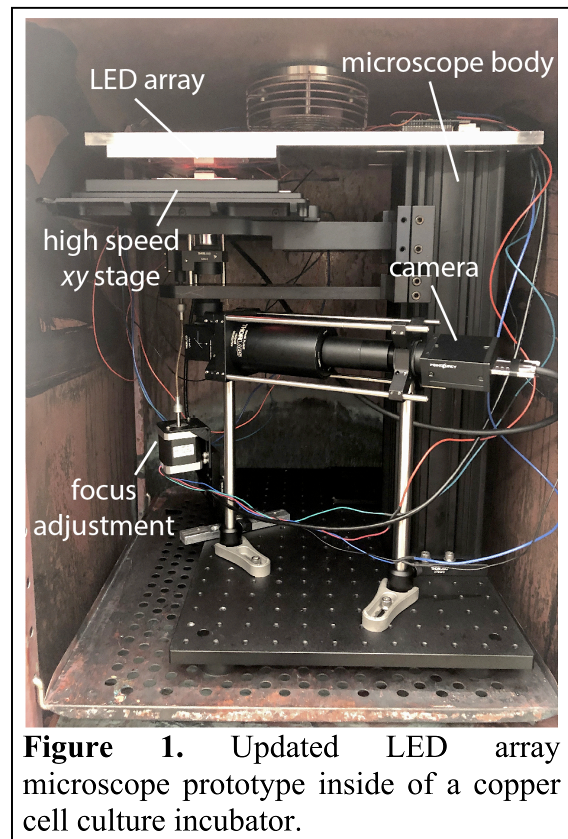

Figure 1. Updated LED array microscope prototype inside of a copper cell culture incubator.

What was accomplished under these goals?

The primary activity (***Zangle***) in year 1 was to build and validate the QPM system described in the proposal and SOW (**Task 1**). As shown in **Figure 1**, the QPM consists of a custom-built microscope with a high-speed scanning *xy* stage. Quantitative phase images are constructed using the differential

phase contrast (DPC) technique (Tian and Waller, *Optics Express* 23(9) 2015, 11395). The entire system was redesigned to fit within a standard cell culture incubator. In accordance with the SOW, QPM hardware and software were optimized to achieve a total time of 0.85 s per frame, under the 1 s per frame target. This includes all imaging, illumination, and *x,y,z* movement operations.

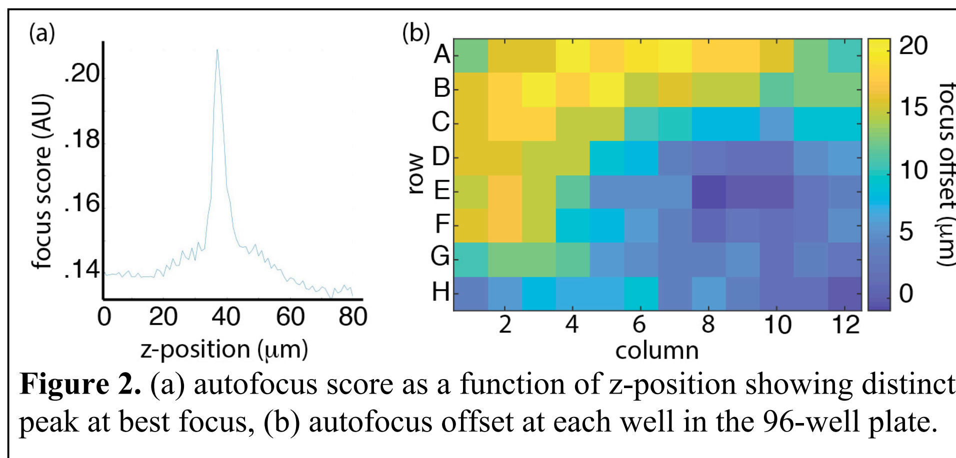

Figure 2. (a) autofocus score as a function of z-position showing distinct peak at best focus, (b) autofocus offset at each well in the 96-well plate.

Autofocus is required for long term imaging experiments to compensate for drift due to thermal gradients. To provide this feature while keeping with our target of less than 1 s per frame, we (***Zangle***) implemented autofocus at a single measurement point per imaging cycle. Our autofocus method is image-based and relies on evaluating sharpness of the text in the corner (near well H12) of the 96 well plate we use for measurements. This method yields a reproducible peak in focus score (**Figure 2a**) which can be found using an optimization routine during each imaging loop. The *z*-position of each measurement is then found based on a predetermined offset from this focus location (**Figure 2b**) to account for variation in the plate plus any tilt of the plate during measurement setup. This method takes 25 s, and is performed once per imaging loop. Across the 864 imaging locations per experiment (9 locations x 96 wells) this means the total autofocus + imaging + motion time per imaging location is 0.88 s, well under the performance target of 1 s per frame (**Task 1.2**).

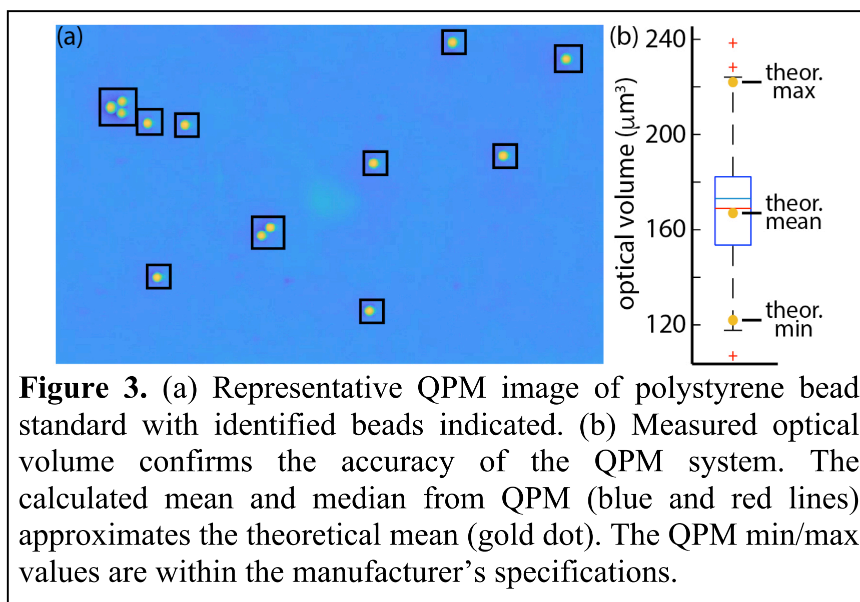

Figure 3. (a) Representative QPM image of polystyrene bead standard with identified beads indicated. (b) Measured optical volume confirms the accuracy of the QPM system. The calculated mean and median from QPM (blue and red lines) approximates the theoretical mean (gold dot). The QPM min/max values are within the manufacturer's specifications.

The next step (***Zangle***) was to validate the system using polystyrene beads. We used 20 μm polystyrene beads (n = 1.59) immobilized in a UV curable optical polymer with n = 1.59 as a stable and reproducible standard for QPM. **Figure 3** shows the results of this analysis. The measured optical volume agreed with the theoretical minimum and maximum values based on manufacturer specified tolerances on size and refractive index on images acquired within less than 1 s per frame (**Task 1.2**).

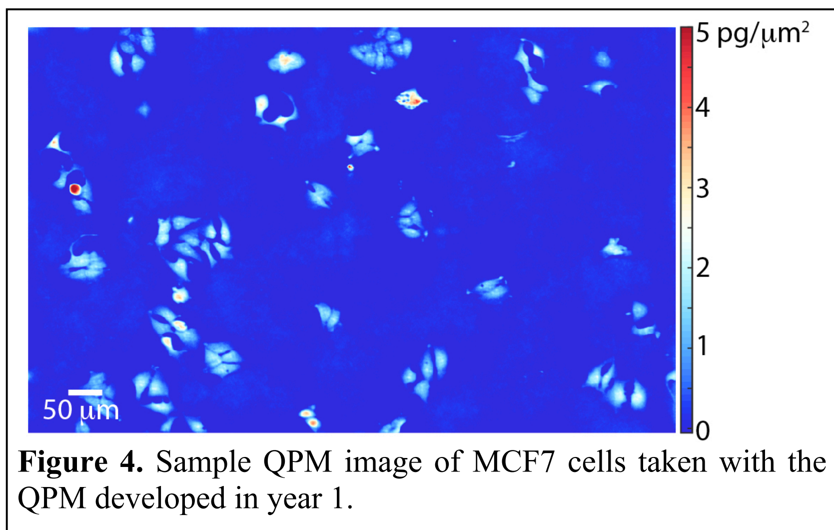

Figure 4. Sample QPM image of MCF7 cells taken with the QPM developed in year 1.

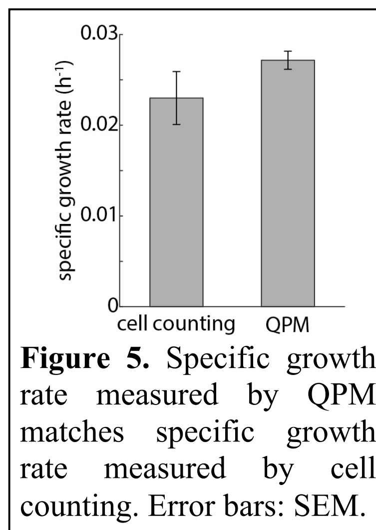

Figure 5. Specific growth rate measured by QPM matches specific growth rate measured by cell counting. Error bars: SEM.

We (***Zangle***) validated our QPM method using MCF7 breast cancer cells (**Figure 4**). Cell morphology and dry mass acquired with our custom-built system are consistent with results obtained using commercial QPM methods. We validated our system against a cell counting growth/proliferation assay (***Bernard***). As shown in **Figure 5**, the QPM results for MCF-7 cell line growth/proliferation agree with results from cell counting (**Task 1.2**). Three additional commercial cell lines (BT-474, SK-BR3, and MDA-MB-231) are currently being expanded for cell counting ***(Bernard)*** and QPM growth/proliferation assays ***(Zangle)***.

The cell lines above have been selected do to their diversity in breast cancer subtypes and biomarker expression: MCF7 (ER+/PR+/HER2-), BT-474 (ER-/PR+/HER2+), SK-BR3 (ER-/PR-/HER2+), and MDA-MB-231 (ER-/PR-/HER2-). Furthermore, these cell lines show a spectrum of response to commonly used chemotherapies and directed therapies used in breast cancer. The Genomics of Drug Sensitivity in Cancer database (https://www.cancerrxgene.org/) has EC50 profiles on over 1000 human cancer cell lines and 100 compounds. We will use this database as a reference for selecting compounds in our QPM drug sensitivity assays and confirm results with our in-house CellTiterGlo assay ***(Bernard*****, Task 2.1**).

Using our QPM system, we performed a 6-point dose response assay using an initial panel of 5 chemotherapies, chosen to have a range of mechanisms of action and responses from CellTiterGlo assay results (**Figure 6, Task 2.2**). For this assay, 9 locations were selected within each well of a 96 well plate, resulting in 864 total images acquired per 15 min imaging loop. This loop was repeated for 20 h of data collection. Therapies were plated at the indicated concentrations in triplicate wells per therapy. Overall, the assay results indicate the expected sensitivities to doxorubicin and fulvestrant. In accordance with **Task 1.2**, the overall computational time was reduced to 10 h by optimization of the image processing methods and acquisition of a dedicated image processing computer for this project.

Finally, we have obtained IRB and HRPO approval for use of human samples as outlined in our proposal (***Bernard***, **Task 3.1**, **3.2**, **Milestone 2**). Patients have been consented for cancer research using our Huntsman Cancer Institute Total Cancer Care (IRB#89989) protocol, which allows for retrospective, de-identified clinical data linkage and analyses. We now have over 50

breast cancers procured from primary and metastatic sites. Many have been grown as patient derived organoids, either from mouse xenografts (PDxO) or directly from the patient (PDO). In collaboration with the Welm lab, we are selecting organoids with different subtypes, activation of different pathways, and different drug sensitivities. These will be grown as 3D organoids for the QPM drug screening.

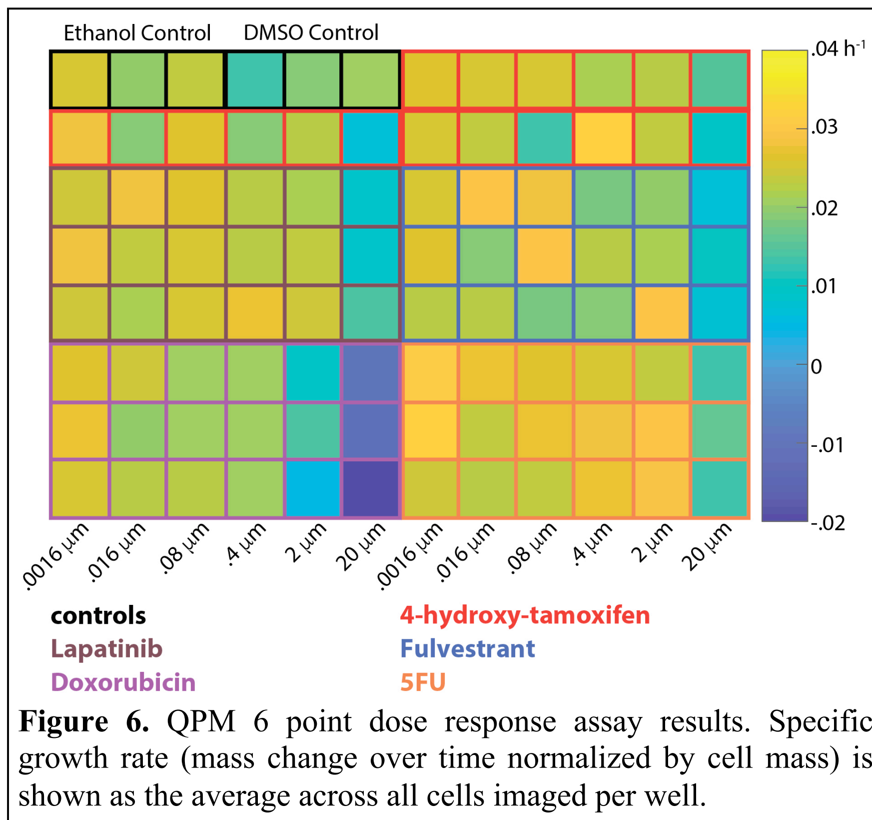

Figure 6. QPM 6 point dose response assay results. Specific growth rate (mass change over time normalized by cell mass) is shown as the average across all cells imaged per well.

Moving towards the eventual transition of the QPM from Zangle lab to Bernard lab (***Zangle/Bernard***), we havebegun monthly meetings among the Zangle, Bernard, and Welm labs. These meetings alternate between the bioengineering and Huntsman Cancer Institute building on the University of Utah campus (or are held via Zoom meeting).

What opportunities for training and professional development has the project provided?

In year 1, this project has resulted in multiple training and professional development opportunities for students in the ***Zangle*** lab and the University of Utah. In terms of mentorship, two graduate students have been involved in this project. Specific mentorship activities include participation in lab workshops on manuscript writing, presentations with feedback at weekly group meetings, and participation in the Department of Chemical Engineering's annual Graduate Research Symposium. These graduate students have also gained experience presenting their research in an interdisciplinary group setting at monthly joint ***Zangle/Bernard*** group meetings and received training by postdocs in the ***Bernard*** and Welm labs. Additionally, one graduate student in the ***Zangle*** lab attended a short course on Fourier Optics at the SPIE Photonics West meeting.

In terms of undergraduate student mentorship, three undergraduate students have worked in the ***Zangle*** lab during the reporting period. These students were supported through the University of Utah Undergraduate Research Opportunities Program (UROP). As part of this program, each student had to prepare a project proposal and statement of work. UROP students are also required to present their work at a public poster session in an annual Undergraduate Research Symposium. A fourth undergraduate student in the ***Zangle*** lab worked on an Honors thesis related to use of QPM for precision medicine. Finally, PI ***Zangle*** also sponsored a project group

(involving the honors thesis student + two additional chemical engineering undergraduates) for a capstone project in chemical engineering (described below).

How were the results disseminated to communities of interest?
This project was incorporated into an undergraduate capstone project class taught by PI ***Zangle***. In this class, groups of chemical engineering seniors are asked to form teams of 2-4 students to solve problems they have identified. As part of the project selection process, in December 2019, PI Zangle presented the concept of precision medicine screening to the senior class in chemical engineering (65 students). One team of three seniors selected this project direction. During the spring semester this team successfully designed and tested a microfluidic device for automatically performing serial dilution, a key step in the process of chemotherapy screening. These students then presented their project results to the class in a series of formal presentations. Additionally, final results of this project were presented at a public (virtual) symposium and poster session held on March 26, 2020.

Additionally, in September 2019 PI ***Zangle*** gave a public research seminar to the University of Utah Math Department. This presentation included the concepts of precision medicine and screening by QPM. The audience included faculty, undergrad students, and graduate students generally knowledgeable about biology, but all working outside the cancer field.

What do you plan to do during the next reporting period to accomplish the goals?
This project is at an exciting phase. With instrument development completed, our (***Zangle***) next primary task is to apply this system to screen our panel of therapies against our panel of cell lines. With the current performance of the QPM system, this represents about 5 months of data collection to obtain triplicate results and planned control experiments. We note that, despite the promise of QPM for rapidly screening chemotherapies, this kind of robust dataset has not been generated before. One factor is the time required for this screen with other systems. We estimate the same dataset would have required 5 years to generate the same depth of data (number of replicates and controls) with a typical QPM.

Using this dataset, we (***Zangle***) will build a rigorous statistical model of QPM performance as outlined in our initial proposal and SOW. This step is essential for future deployment as a clinical test.

During the cell line testing phase, ***Zangle*** lab members will train ***Bernard*** lab members on the use of QPM. This will move the project towards use in a clinical setting and also identify any remaining technical issues that need to be addressed. ***Bernard*** lab will also finish collection of data for cell lines using gold standard approaches (cell counting and CellTiter Glo).

Moving towards clinical samples, ***Bernard*** lab will work on patient sample preparation for use with QPM. By the end of the next reporting period, we (***Zangle/Bernard***) plan to select patient samples and begin response testing with QPM.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Cancer patients with advanced disease and no available treatment options will often have their tumors analyzed using genetic techniques. This is expensive and usually does not identify better therapies. For these patients, there is no quantitative way to predict how their cancer will respond to therapy fast enough to help doctors choose the best option. Our project uses a new approach based on weighing single cancer cells growing outside the body with light. By weighing cancer cells as they increase in size over time, we directly measure growth. We aim to use this method to observe a patient's cancer cells when treated with possible therapies. This will show us which therapies stop or slow cancer cell growth, suggesting they will likely be effective in the patient. Our overall goal is to be able to give a personalized drug sensitivity/resistance report back to the doctor within 2 d.

The time required by our method is essential. During the first year, we built and tested an instrument that is fast enough to measure therapy response within hours. We demonstrated that our device is accurate enough for our proposed test and showed that it can measure the response of cancer cells grown in culture to commonly used therapies. The overall number of therapies that this instrument can screen is a significant improvement over the previous state of the art.

In the future we will use this instrument to test many different therapies so we can provide options for the most appropriate treatment. Then we will then see how well our approach does with cancer cells collected directly from patients with advanced or metastatic breast cancer who have been treated at the Huntsman Cancer Institute. We will compare our results to the known clinical outcome of each patient whose samples we tested. This will tell us how good our method is at predicting when a patient is likely to respond or not to a given therapy.

The overall potential impact of our project is to reduce patient suffering by reducing unnecessary side effects of ineffective treatments. We also hope to improve survival of breast cancer patients by telling doctors which therapy to use for each individual patient. We have chosen to start with patients who have advanced or metastatic breast cancer because we have the samples available at our institution and this patient population could benefit most from our testing. These patients have typically been through multiple rounds of treatments which allows us to compare our test predictions to how these patients responded in the past. However, the basic idea of our method could be applied to samples from any breast cancer. Ultimately, our approach will support the BCRP's mission of ending breast cancer by allowing doctors to give the right treatment to the right patient at the right time.

What was the impact on other disciplines?

The instrument and methods developed in this project have the potential to apply broadly outside of breast cancer research. This general approach developed in this project can be applied to other solid and blood tumor types. The QPM method and instrument developed in this project period can also be applied to study other basic biological processes that impact human health in immunity, infectious disease, and aging. Among these, PI Zangle has a currently funded sub-project through the NIH/NIAID studying B cell development via QPM that could benefit from the approach developed under this award.

What was the impact on technology transfer?
One of the principal investigators (***Zangle*** – initiating award PI) for this project is an engineer who helped develop the method we plan to use. The other principal investigator (***Bernard*** – sub-award PI) is a board certified clinical pathologist that serves as a Medical Director for a large pathology reference laboratory that offers cancer testing using methods that he developed and validated. PI Bernard has also previously developed and commercialized a diagnostic test for breast caner. As a team, we plan to move this promising technology, based on the results of year 1 of this project, into a method that can help patients within the next five years.

What was the impact on society beyond science and technology?
The overall goal of this project remains changing the way decisions are made for patients with advanced metastatic disease. The results of this project, which build on the instrument development work done in year 1, could change the public perception of chemotherapy by making this a more focused approach with fewer side effects due to ineffective treatments.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change
We have also moved to a 6 point dose response (+ control) instead of 5 (+ control) to better align with this data and methods currently used in Welm lab CellTiterGlo experiments. Welm lab uses an 8 point dose response at 5-fold dilutions per step. With 6 points we are able to cover approximately the same range using 5-fold dilution in each central step with 10 fold dilutions to the high and low concentration extremes. Together with the new list, this will enable us to generate a complete set of dose-response data in triplicate wells using 5 96-well plates, or a minimal set (triplicate wells at a single concentration per drug) on a single 96-well plate.

Finally, to better leverage collaborator Welm lab's expertise in culturing primary patient cells, we have decided to test cells from organoids as an intermediate step between cell lines and pleural effusion samples. This will give us more data to directly compare to existing gold standard datasets and validate our plating approach before moving to pleural effusion samples.

Actual or anticipated problems or delays and actions or plans to resolve them
The move to a 6 point dose response assay will moderately more QPM experiments. The QPM developed in year 1 can perform a single trial in one day, so our overall throughput is sufficient for this. We have also built a dedicated computer that can process data in 11 h so we can keep up with QPM data generation without delays for analysis. The addition of organoid testing will require more testing of patient samples, but we plan to compensate with shared responsibility for running QPM (***Zangle/Bernard***) experiments in later stages of the project.

Changes that had a significant impact on expenditures
There was a delay in hiring a postdoc- for the Bernard lab due to a lack of suitable candidates with the required expertise in growing cancer organoids and performing drug screens.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects
Nothing to Report.

Significant changes in use or care of vertebrate animals
Nothing to Report.

Significant changes in use of biohazards and/or select agents
Nothing to Report.

6. PRODUCTS

Publications, conference papers, and presentations:

Journal publications.
Nothing to Report.

Books or other non-periodical, one-time publications.
Nothing to Report.

Other publications, conference papers and presentations.
Polanco, E.R., Griffin, J., Western, N., and Zangle, T.A., "Low refractive index microfluidic device fabrication for quantitative phase imaging," SPIE Photonics West, San Francisco, CA, February 2-7, 2019.

Website(s) or other Internet site(s)
The scope of this project was described for the public at:
https://zanglelab.che.utah.edu/letter-of-hope/
This information was publicized by the Huntsman Cancer Institute at the University of Utah as part of their Letter of Hope campaign to raise awareness about ongoing research activities.

Technologies or techniques
During the reporting period we (***Zangle***) developed a QPM system for dedicated screening of chemotherapies as described in the original project proposal.

Inventions, patent applications, and/or licenses
Nothing to Report.

Other Products
During this first year we developed:
- The QPM instrument as described above and in the original project proposal.
- Data on MCF7 response to a preliminary set of chemotherapies that will guide future application of QPM for chemotherapy screening.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Zangle (BC180931)

Name: Thomas Zangle, Ph.D.
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): ORCID: 0000-0001-5899-3517
Nearest person month worked: 1
Contribution to Project: Dr. Zangle co-directed the research project including experiment planning, data analysis, QPM development, presentations and personnel supervision.
Funding Support: NIH/NIAID and University of Utah

Name: Edward Polanco, B.S.
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): ORCID: 0000-0001-7388-962X
Nearest person month worked: 11
Contribution to Project: Eddie worked on QPM instrument development, cell culture, and preliminary drug screening.
Funding Support: N/A

Name: Tarek Moustafa, B.S.
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): ORCID: 0000-0001-5282-8246
Nearest person month worked: 4
Contribution to Project: Tarek worked on statistical modeling of QPM data and calibration of the instrument.
Funding Support: N/A

Name: Kenneth Boucher, Ph.D.
Project Role: Consultant
Researcher Identifier (e.g. ORCID ID): ORCID: 0000-0003-2833-0127
Nearest person month worked: 1
Contribution to Project: Dr. Boucher provided support for statistical analysis and power calculations for human subject testing.
Funding Support: NIH/NCI, NIH/NCATS, University of Utah, Pfizer Inc., American Cancer Society, Susan B. Komen Foundation

Bernard (BC180931P1)

Name: Philip Bernard, M.D.
Project Role: Principal Investigator (partner award)
Researcher Identifier (e.g. ORCID ID): ORCID: 0000-0002-1418-8521
Nearest person month worked: 4
Contribution to Project: Dr. Bernard led efforts to secure IRB approvals for specimen handling, oversaw personnel performing "gold

standard" measurements, and performed data analysis and project planning.

Funding Support: NIH/NCI

Name: Bryan Welm, Ph.D.
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): ORCID: 0000-0002-1879-6612
Nearest person month worked: 1
Contribution to Project: Dr. Welm provided expertise in developing a clinical drug screen, working with patient derived cells, and selection of therapies for the QPM screening demonstration.
Funding Support: NIH/NCI

Name: Byeong-Il Kang, Ph.D.
Project Role: Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID): ORCID: 0000-0001-6208-501X
Nearest person month worked: 1
Contribution to Project: Dr. Kang expanded cell lines for use with the QPM drug screen and performed initial validation of the cell assays.
Funding Support: N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report.

What other organizations were involved as partners?
Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
Tasks and personnel specific to the prime award, ***Zangle*** BC180931, and the sub-award, ***Bernard*** BC180931P1 have been indicated in the report above.

9. APPENDICES:

Nothing to Report.