AD_____

AWARD NUMBER: W81XWH-17-1-0011

TITLE: Understanding the Role of Matrix Microstructure in Metastasis

PRINCIPAL INVESTIGATOR: Edward Brown

RECIPIENT: University of Rochester
Rochester, NY 14642

REPORT DATE: Feb 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
Feb 2019	Annual	1 Feb 2018 - 31 Jan 2019

4. TITLE AND SUBTITLE
Understanding the Role of Matrix Microstructure in Metastasis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0011

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Edward Brown and Catherine Kuo

E-Mail:edward_brown@urmc.rochester.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Department of Biomedical Engineering
University of Rochester
201 Robert B. Goergen Hall
P.O. Box 270168
Rochester, NY 14627

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

In this project we will address the underlying mechanisms by which certain light-scattering properties of the tumor ECM predict metastasis. Understanding the mechanisms of this novel phenomenon may yield novel insights into metastatic processes, leading to new treatments for metastatic breast cancer. It may also uncover additional prognostic indicators, improving our predictive ability and further reducing overtreatment. The light scattering phenomenon in question is the direction that second harmonic generation light scatters from collagen fibers, the "F/B ratio". F/B is sensitive to the diameter of fibrils that are bundled into collagen fibers, as well as the spacing and disorder of their packing within the fiber, altogether known as a fiber's "microstructure". To begin to address why collagen microstructure, reported by F/B, predicts patients' metastatic outcome, we must first determine how that microstructure is defined by cells within the tumor, and the cues that influence those cells to do so.

15. SUBJECT TERMS
Microscopy, metastasis

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1.INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Overtreatment of breast cancer is a pressing clinical problem as patients are subjected to the toxic side effects of chemotherapy even though they were not going to experience any post-surgical metastases. We recently discovered an innovative method to predict metastatic outcome in invasive ductal carcinoma (IDC) using light scattering from tumor collagen as an independent prognostic indicator. In this project we will address the underlying mechanisms by which these light-scattering properties predict metastasis. Understanding the mechanisms of this novel phenomenon may yield novel insights into metastatic processes, leading to new treatments for metastatic breast cancer. The light scattering phenomenon in question is the direction that second harmonic generation light scatters from collagen fibers, the "F/B" ratio. F/B is sensitive to the diameter of fibrils that are bundled into collagen fibers, as well as the spacing and disorder of their packing within the fiber, altogether known as a fiber's "microstructure". To begin to address why collagen microstructure, reported by F/B, predicts patients' metastatic outcome, we must first determine how that microstructure is defined by cells within the tumor, and the cues that influence those cells to do so. We hypothesize that tumor cells and fibroblasts respond to presented matrix cues, including collagen subtypes, crosslink density, and the presented microstructure itself, to modify collagen microstructure (and hence F/B) by digestion of existing fibrillar collagen with MMPs, synthesis of fibrillar collagen, and LOX establishment of new crosslinks. This project will significantly impact the fight against breast cancer because understanding the mechanisms by which F/B predicts metastasis may uncover additional metastasis predictors, which will improve our ability to identify who should receive adjuvant chemotherapy. Improved predictive formulas can be incorporated into our ongoing development process and rapidly moved towards the clinic, thereby accelerating and enhancing the impact. Mechanistic understanding may also yield novel insights into metastatic processes. We are exploiting a novel way to study metastatic processes (by exploring the role of collagen microstructure) and thus newly discovered mechanisms affecting metastasis will likely themselves be novel. We will then be poised to explore druggable targets for antimetastatic therapies based upon these insights.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Metastasis, collagen, microenvironment, microscopy

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project? *List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*

The Specific Aims of this three-year grant are as follows, and are unchanged from the original submission:

Aim 1: Determine how F/B is related to collagen properties in IDC ER+ LNN primary tumors.
- 1a. Evaluate F/B vs collagen I:III and I:V ratios in IDC tumor samples.
- 1b. Evaluate F/B vs collagen crosslink density in IDC tumor samples.

Aim 2: Determine what parameters influence F/B modification activity by the cells in a primary tumor.
- 2a. Alter collagen ratios (I;III and I:V) in collagen gels and quantify resultant F/B modification by tumor cells and by fibroblasts.
- 2b. Alter crosslink density in collagen gels and quantify resultant F/B modification by tumor cells and by fibroblasts.
- 2c. Test mechanisms by repeating 2a,b after inhibiting formation of new crosslinks, collagen synthesis, and MMP activity.
- 2d. Repeat 2a-c in decellularized tumor tissue.

Aim 3: Expand our ability to predict metastatic outcome in patient biopsy samples.
- 3a. Add quantification of collagen I:III and I:V ratio, and crosslink density, to our predictive formula, and assess its predictive strength.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

This project began with the observation that a light-scattering phenomenon, specifically the directionality of second harmonic generation quantified as "F/B", is prognostic of metastasis free survival time in untreated patients with invasive ductal carcinoma (IDC) that are estrogen receptor positive (ER+) and do not yet have significant visible signs of tumor cells in their lymph nodes (N0). The ultimate goal of the work is two-fold: 1) to propel that observation into a clinically useful tool for patients that will facilitate patient-tailored therapy by identifying who should, and should not, receive chemotherapy, and 2) to study the mechanisms underlying the relationship between F/B and metastasis in order to learn more about the metastatic process and hence develop anti-metastatic therapy. Aims 1 + 2 of the grant address goal #2, while Aim 3 addresses goal #1. To accomplish goal #1 broadly speaking we wish to first confirm our results (that F/B predicts metastasis) in a US-based population with the modern distribution of neoadjuvant and adjuvant chemotherapy (the original discovery was made with a Dutch-based population who received neither adjuvant nor neoadjuvant chemotherapy), evaluate additional related predictors such as Col I/III immunolabeling, then ask the more direct question if F/B plus related predictors can predict who will, and will not, respond to chemotherapy, then generate and validate the appropriate predictive model, then move to a multicenter trial to test that predictive model. While accruing patients with curated samples and data sets for Aim 3 as described in item 6 below, we were approached by a clinical collaborator with

an exciting and highly relevant *pre-existing* data set: a set of formalin-fixed paraffin-embedded needle biopsy sections with the associated patient data denoting subsequent response to neoadjuvant chemotherapy recorded as the Residual Cancer Burden (RCB) score. This sample set allows us to rapidly gain insight into the question as to whether F/B, in addition to predicting metastasis, can also predict response to chemotherapy, albeit strictly in the neoadjuvant setting. This allows us to "skip a step" and see if F/B predicts chemoresponse as well as metastatic outcome, again with the caveat that it asks that question in the neoadjuvant setting. Once we have accomplished our accrual of our full data set of archived samples from primary tumor excision and associated data on neoadjuvant and adjuvant chemotherapy (described in item 6 below), we can expand our study to the adjuvant setting and also expand our study numbers. I will describe our progress with this new neoadjuvant data set as my first numbered point below, then continue with numbered updates of other progress on this project:

1) For Aim 3 (Performed in Brown Lab): Breast cancer patients with IDC who receive neoadjuvant chemotherapy primarily fall into two groups: HER2+ and triple negative (TNBC). We have performed F/B assessment on these specimens in our usual manner (Burke et al. 2013, 2015). In brief, SHG images in the forward (F) and backward (B) direction are generated for each field of view. A blinded observer selects an intensity-based threshold for all "F" and all "B" images in the data set (i.e. one threshold for each of the two sets of images) which sets pixels to "1" if they are within collagen fibers and sets them to "0" if they are within background regions. These two masks are multiplied together to create a final intensity-based mask to select collagen pixels for study. This mask is then multiplied by all F/B ratio images and the average value of all nonzero pixels is calculated to determine the average F/B value of the imaged region. Using this method, F/B is calculated from 3 fields of view in the tumor/host interface, as well as 3 fields of view in the tumor bulk, of needle biopsy sections from HER2+ and TNBC patients and compared the results to the subsequent RCB score. RCB scores of 0, 1, 2, 3, and 4 correspond to progressively poorer response, and patients whose RCB scores are 0 or 1 (complete or significant response, respectively) have the same clinical outcomes. Therefore, we pooled RCB scores of 0 and 1, as well as RCB scores of 2 and 3 for this study. In HER2+ patients, *we find that there is a statistically significant correlation between F/B and RCB score category when F/B is assessed in the tumor/host interface* (Figure 1a) but not in the tumor bulk (Figure 1b). In TNBC patients, there is no significant relationship between F/B and RCB category in either tumor region (Figure 2). We have also evaluated the predictive ability of F/B when pixels are selected based upon their "coherency", a measure of the linearity of nearby pixels. This selects pixels that lie in linear structures versus simply selecting bright pixels. Interestingly, when pixels are selected based upon their coherency there is no predictive ability of F/B in any of the samples or regions studied (data not shown), suggesting that collagen whose microstructure facilitates strong SHG signal is more relevant to metastatic outcome versus collagen which is bunched into clear fiber networks.

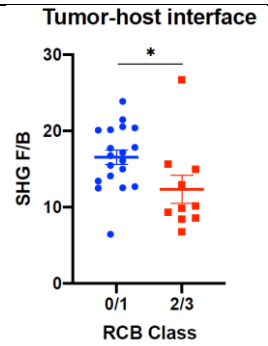
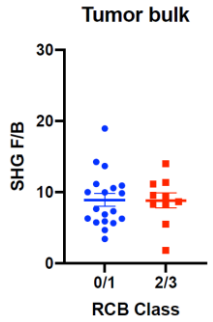
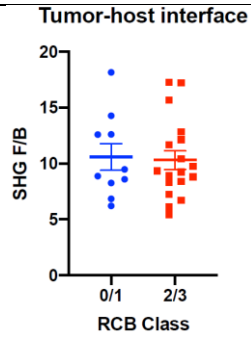
		

Figure 1a) F/B versus Residual Cancer Burden (RCB) score in HER2+ needle biopsy sections. Measured at the tumor/host interface. p=0.03, N=29.	Figure 1b) F/B versus RCB score in HER2+ needle biopsy sections. Measured in the tumor bulk. p>0.05, N = 29.	Figure 2a) F/B versus RCB score in TNBC needle biopsy sections. Measured at the tumor/host interface. p>0.05, N=28.

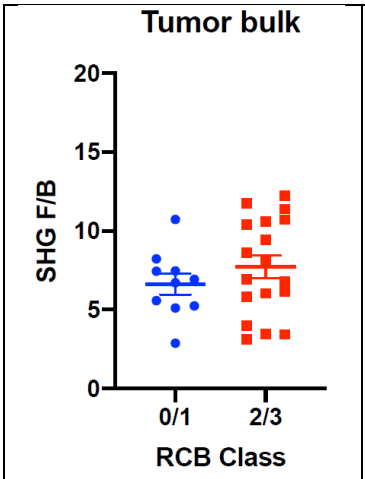
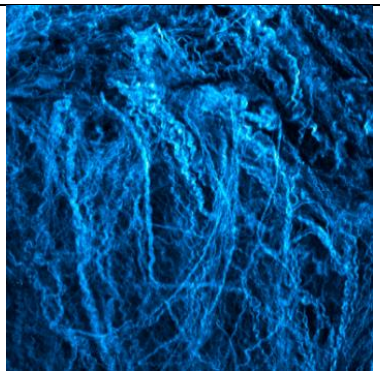
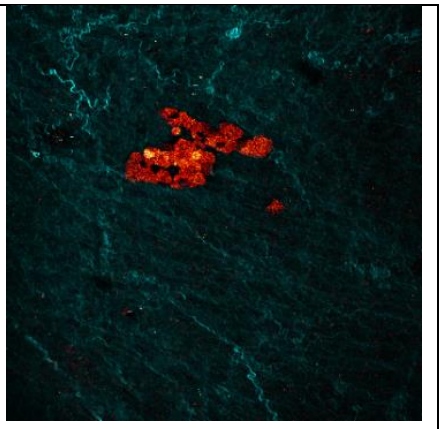
		

Figure 2b. F/B versus RCB score in TNBC needle biopsy sections, measured in the tumor bulk. p>0.05, N=28.	Figure 3. Decellularized tumor from the mouse mammary fat pad. Backward scattered SHG image, image is 660 μm across.	Figure 4. SHG and two-photon fluorescence from de-cellularized murine mammary tumor one day post-reseeding with tdTomato-transfected 4T1 tumor cells. Image is 660 μm across.

2) <u>For Aim 2 (performed in Brown Lab):</u> We secured IRB approval for acquiring fresh human tumor specimens for Aim 2, and are now routinely performing decellularization of those specimens and characterization of the resultant matrices. While awaiting IRB approval we first optimized our decellularization methods on murine tumors (Figure 3). We also optimized our pCR analysis methods and determined that our decellularization protocols were removing all detectable DNA from the matrices, with a typical average DNA concentration of 1.30+/-0.13 ng/mL where the literature-accepted criterion for successful decellularization is <50 ng/mL and our non decellularized controls typically exhibited DNA concentrations >1050 ng/mL. We also successfully reintroduced fluorescently labeled "probe" cells into the decellularized murine tumor matrices and observed their incorporation into the matrix (Figure 4). Upon IRB approval we began securing

fresh human specimens, decellularizing them, verifying decellularization by pCR analysis (DNA concentrations of 0.21+/- 0.2 ng/mL where the accepted criterion is <50 ng/mL and non decellularized samples were again >1050 ng/mL). This is now routine in the lab. We have SHG imaged them (Figure 5), and found that occasionally we would produce decellularized samples that were "optically thick", meaning that scattering of SHG light by the sample was sufficient to make F/B vary with depth. It became necessary to develop a protocol for assessing "optical thinness" of the samples, and a quantitative criterion for rejecting some samples as "optically thick" based upon the slope of F/B versus depth (Figure 6).

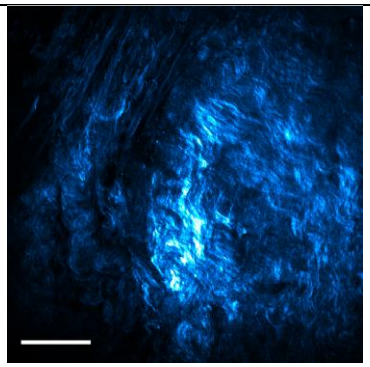
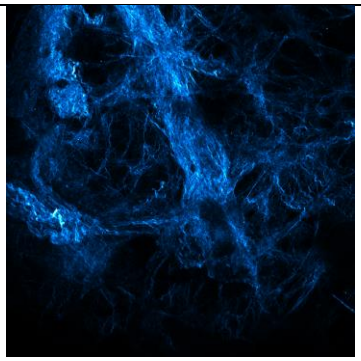


Figure 5a (left). SHG image of decellularized human IDC tumor. Scale bar is 200 μm. Figure 5b (right). SHG image of decellularized human IDC tumor. Tumor from two different patients exhibit different collagen structures. Image is 600 μm across.



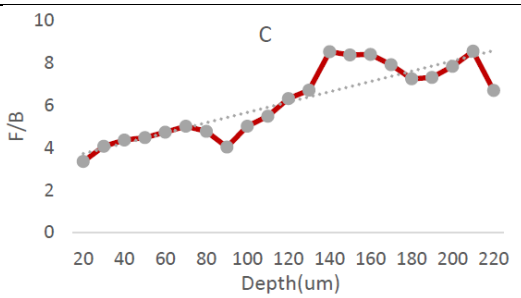


Figure 6a. F/B versus depth for a decellularized IDC sample which is optically thick, exhibiting a statistically significant variation of F/B with depth using a linear fit model.

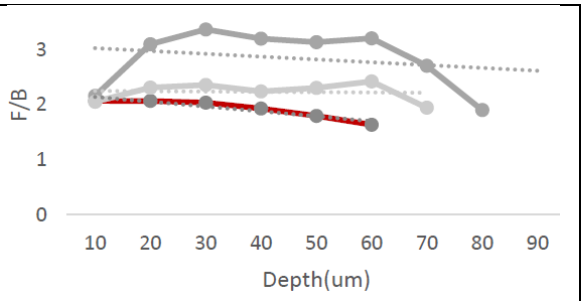


Figure 6b. F/B versus depth for a two decellularized IDC samples which are optically thin based upon our allowed range of slopes of F/B vs depth (gray), and one that is disallowed (red).

3) For Aim 3 (performed in Brown Lab): Based upon our results on needle biopsy samples described in item 1 above, in which F/B was predictive of chemoresponse *if assessed in the tumor-host interface* but not if assessed in the tumor bulk, we felt it necessary to explore if there was also a location-specific effect on F/B's ability to predict metastatic outcome. Our original discovery (i.e. that F/B predicts metastasis) was produced with a tissue microarray of 125 untreated ER+ Dutch patients. From our Dutch collaborators we secured full tissue sections from 96 of those patients and quantified F/B in 3 locations in the tumor-host interface as well as 3 locations in the bulk of the tumor, as well as 3 locations as far from the tumor as possible (it was not always possible to find 3 locations not contained in, nor adjacent to, tumor tissue). F/B was determined in our usual way, by a blinded user selecting intensity thresholds to reject background pixels, etc. We found that F/B was predictive of metastasis when measured in the tumor/host interface (Figure 10), in

the tumor bulk (Figure 11), but not when measured far from the tumor (data not shown). Interestingly, the p value for the tumor/host interface measurement was significantly smaller than that for the tumor bulk, consistent with our findings in item 1 above that F/B is predictive of chemoresponse when measured in the tumor/host interface but not in the tumor bulk (Figure 1). When sample accrual is complete in Aim 3 we will be sure to assess both areas of the tumor.

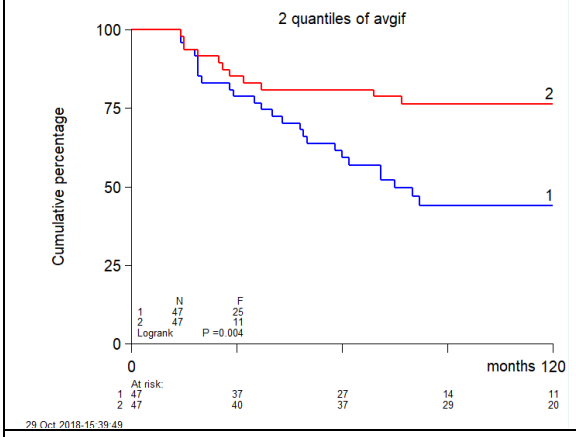


Figure 10. Kaplan-Meier plot of metastasis-free survival versus time for two cohorts of patients who were grouped by the F/B value measured in 3 locations in the tumor/host interface. p=0.004.

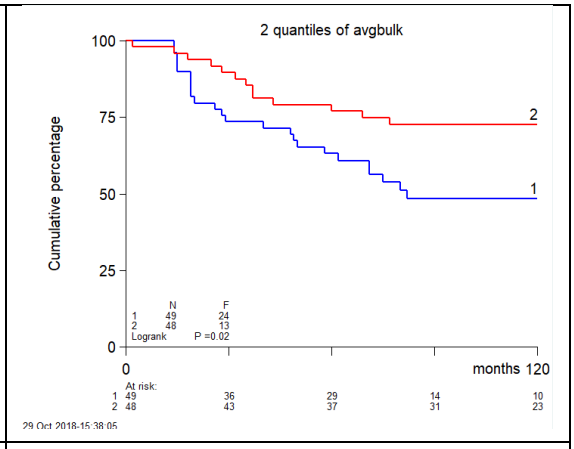


Figure 11. Kaplan-Meier plot of metastasis-free survival versus time for two cohorts of patients who were grouped by the F/B value measured in 3 locations in the tumor bulk. p=0.004.

4) <u>For Aim 2 (performed in Kuo Lab):</u> The ultimate goal of this aim is to produce collagen gels of varying F/B in a thin layer atop a polyacrylamide (PA) gel to enable F/B (dictated by the collagen) to be modulated independently of stiffness (dictated by the PA). The first requirement is to be able to vary F/B in a controlled manner, an ability that has escaped quite a few students after our original demonstration of several methods to accomplish this (Burke et al 2015). Thanks to a neat, careful, and precise student, we can again routinely vary F/B in collagen gels in a controlled manner, by varying pH, ionic strength, and collagen I/III ratio during polymerization (Figure 13). We began this work last year by generating mixed (but not two-layer) gels of collagen and polyacrylamide, as reported in the previous report. We can now reproducibly make two-layer collagen/PA gels (Figure 14) and have validated their elastic modulus via AFM (Figure 15) although we are still having some minor technical problems with the gels occasionally peeling apart, and may eventually move back to mixed (but not two-layer) gels if we believe that will speed up our work.

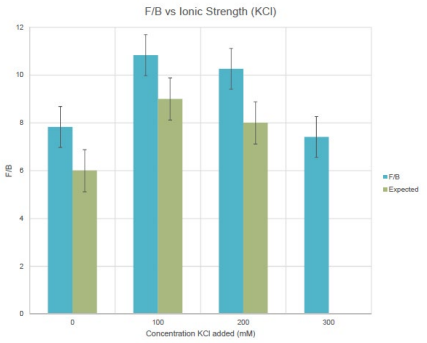


Figure 13a. F/B can be controlled via varying ionic strength during gel polymerization.

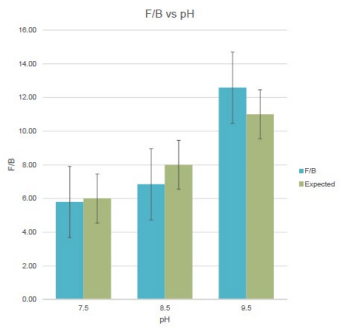


Figure 13b. F/B can be controlled by varying pH during polymerization.

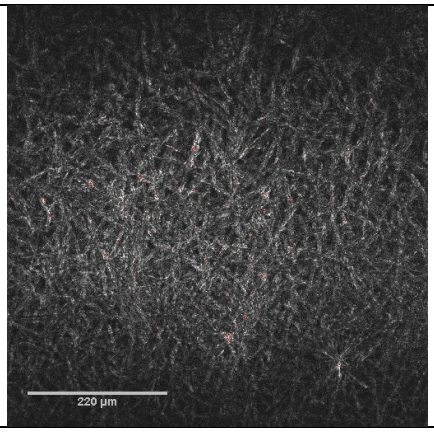


Figure 14. SHG images from the upper collagen layer of two-layer collagen-polyacrylamide gels, in which F/B is controlled by the top layer and elastic modulus by the bottom.

5) <u>For Aim 1 (performed in Kuo Lab):</u> We have now added immunolabeling of collagen V in formalin fixed paraffin embedded sections from patient's tumors to our arsenal (Figure 15), in addition to imunolabeling of Collagen I and III as reported last year. We have also optimized our procedures for co-staining with multiple labels (Figure 16).

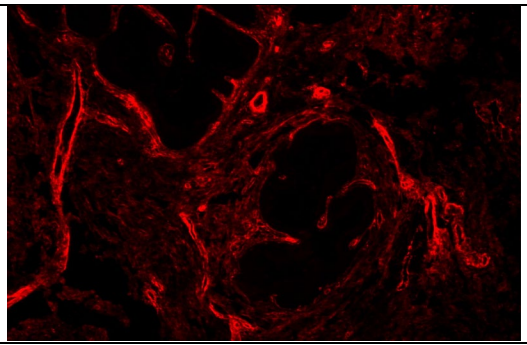


Figure 15. Collagen V staining in formalin fixed paraffin embedded archived tissue sections. Rabbit anti-Human Collagen V Biotinylated + Avidin DL650.

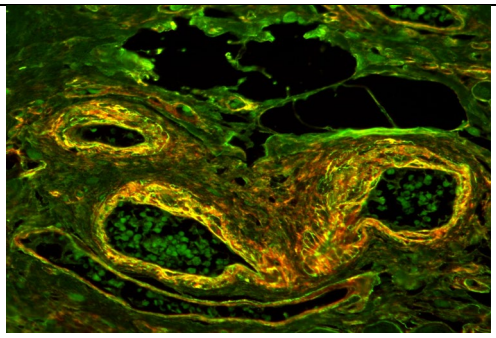


Figure 16. Two-color staining for Collagen I (green) and Collagen V (red) in formalin fixed paraffin embedded archived tissue sections.

6) For Aim 3 (Performed in Brown Lab): We have continued to accumulate archived patient sections and accompanying clinical data for this part of the study, with data validated for 105 patients and sections made for 10 of them. We do not wish to start imaging these samples until all 235 (number based upon power analysis of pilot data) have been accumulated to minimize the danger of day-to-day variability in our imaging system (i.e. we want to image everything all at once), so we cannot report imaging results for them yet.

project progresses to completion, the emphasis in reporting in this section should shift from

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We expect to finish Aim 1 and determine how F/B is related to collagen properties in IDC tumors. We expect to finish Aim 2 and gain insight into tumor cell modulation of microstructure in mixed collagen/PA gels as well as in decellularized tissue. We also expect to finish sample acquisition for Aim 3 and analyze the predictive power of F/B in those samples alone and in combination with immunolabeling for Collagen 1, 3, and 5.

4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to Report. One manuscript is in preparation at this time but has not yet been submitted.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Desa D, Turner B, Buscaglia B, Hill R, Majeski J, Choe R, Strawderman R, Kuo C, Hicks D, Brown E. Using multiphoton laser-scanning microscopy to assess neoadjuvant therapy outcome in core needle biopsies: a novel methodology. San Antonio Breast Cancer Conference. December 2018

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Hill R, Brown E, Hicks D, Buscaglia B, Turner B, Desa D. (2019) A Method to Predict Response to Neoadjuvant Chemotherapy. Provisional Patent Application 62/795,828.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name:	Edward Brown
Project Role:	co-PI
eraCommons:	EBBROWN
Nearest person month worked:	1
Contribution to project:	Dr. Brown has provided general oversight in all aspects of data generation and analysis with particular focus on imaging as well as planning, writing, revising, and submitting IRB protocols.
Name:	Catherine Kuo
Project Role:	co-PI
eraCommons:	ckuo01
Nearest person month worked:	1
Contribution to project:	Dr. Kuo has provided general oversight in all aspects of data generation and analysis with particular focus on mixed gel generation/analysis and crosslink density analysis.

Continued on Next Page:

Name: Bradley Turner
Project Role: co-Investigator
eraCommons BMTMDMPH
Nearest person month worked: 1
Contribution to project: Dr. Turner has assisted with sample assessment, archived sample acquisition, and overall clinical relevance of the project.

Name: Rachel Farkas
Project Role: co-Investigator
eraCommons N/A
Nearest person month worked: 1
Contribution to project: Dr. Farkas assists with acquisition of human tumor samples and overall clinical relevance of the project.

Name: Robert Strawderman
Project Role: co-Investigator
eraCommons STRAWDERMAN
Nearest person month worked: 1
Contribution to project: Dr. Strawderman assists with statistical analysis of data and project planning.

Name: Armen Soukazian
Project Role: Technician
eraCommons N/A
Nearest person month worked: 1
Contribution to project: Mr. Soukazian assists with mining of patient records, securing archived patient samples, and interfacing with Dr. Turner.

Name: Danielle Desa
Project Role: Graduate Student
eraCommons N/A
Nearest person month worked: 12
Contribution to project: Ms. Desa has assisted with decellularization procedures, collagen gel creation, immunolabeling, imaging, and creation of a curated human samples dataset.
Funding Support: Wilmot Cancer Institute, University of Rochester

Name: Tresa Elias
Project Role: Undergraduate Student
eraCommons N/A
Nearest person month worked: 2
Contribution to project: Ms. Elias has assisted with collagen gel creation and AFM analyses.
Funding Support: Worked for class credit.

Name:	Sabrina Pan
Project Role:	Graduate Student
eraCommons	N/A
Nearest person month worked	9
Contribution to project:	Ms. Pan has been developing and optimizing atomic force microscopy measurements and imaging techniques to evaluate tissue stiffness, collagen crosslinking, and collagen morphology of tissues.
Name:	Jiewen Li
Project Role:	Graduate Student
eraCommons	N/A
Nearest person month worked	12
Contribution to project:	Ms. Li has been collaborating with Ms. Pan to collect sample tissues for protocol optimization, and has been developing gel fabrication protocols.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.