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TITLE: Identification of Novel Features to Assess Risk and Improve Therapeutic Decision Making for Prostate Cancer Through a Novel High-Parameter Imaging System

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14. ABSTRACT

About 15% of men will be diagnosed with prostate cancer and many will face difficult treatment decisions. This project's goal is to develop and apply a novel imaging strategy that increases the information and potential clinical utility from prostate biopsy samples. Combining two high-dimensional imaging methods: antibody-based imaging mass cytometry (IMC), and mass spectroscopy imaging (MSI) that are layered together, the goal is to generate exceptionally detailed maps of the cells, molecules, and structures from tumor samples. These layered multi-modal images can be used to identify markers that improve assessments of risk and may ultimately inform clinical decisions. In the first year of the project, despite challenges resulting from the COVID19 pandemic, we are nearing completion of method development and optimization studies, generated a tissue microarray containing (TMA) more than 80 prostate cancer biopsy samples, and have nearly completed imaging of the TMA by MSI. Work on applying the IMC platform to the TMA is ongoing. However, using data generated to date, significant progress was made in developing a novel computational workflow to integrate and analyze high-parameter imaging data. The technical information, methods, and protocols generated will be of value to completing the aims of this study, as well as to the larger scientific community. Similarly, the completed analysis framework will address a previously unsolved challenge and advance the application of cutting-edge imaging methods to identify biological correlates. These methods and resulting insights aim to ultimately improve outcomes for cancer patients.

15. SUBJECT TERMS
Prostate cancer, imaging, biopsy, prognostic, tissue microarray, mass spectroscopy, imaging mass cytometry, computational analysis

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1.**INTRODUCTION:**

The overall goal of this research program is to identify specific targets that can be measured to inform treatment decisions and improve outcomes for patients with prostate cancer. The scientific objective of this project is to improve benefit of tissue biopsy for prostate cancer patients by applying a novel combination of two high-parameter imaging methods to increase the information and clinical value derived from biopsy tissues. The first method, mass spectroscopy imaging, is a label free method that can measure 100s to 1000s of analytes based on individual mass to charge (m/z) ratios. The second method, imaging mass cytometry, detects >40 targets labeled with metal conjugated antibodies. The two imaging methods are applied to the same tissue the data can be combined or layered together to generate highly detailed analyses that map cell populations, bio-molecules and drugs within a prostate tissue biopsy specimen. This project specifically aims to apply this comprehensive imaging method to a repository of clinically annotated prostate cancer tissues to identify correlates that improve the discrimination of prostate cancer grade, risk of progression, and likelihood of recurrence. Development of this layered imaging methodology will require optimization of protocols and technical parameters, as well as novel computational tools to directly link data from both imaging methods and conduct statistical analyses that link measured features to clinical data. The initial project period focused on foundational aspects of the work, including securing approvals, sample identification and annotation, and method development. Despite challenges resulting from the COVID19 pandemic, work completed during this period will improve efficiency and quality of the studies to meet the scientific objectives of the grant.

2.**KEYWORDS:** Prostate, cancer, biopsy, grade, recurrence, progression, prognostic, tissue microarray, imaging mass cytometry, mass spectroscopy imaging

3.**ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

Major Task 1: Obtain USAMRMC HRPO approval for use of deidentified prostate biopsy samples

Subtask 1: Obtain necessary IRB documentation demonstrating Dr. Reeves is approved to use de-identified prostate cancer (PC) samples

Subtask 2: Submit necessary documentation to enable HRPO review of MGH IRB approvals for PC samples

Subtask 3: Response to comments and questions from HRPO review

Major Task 1 and all Subtasks were completed in March 2020, ahead of the anticipated month 4.

Specific Aim 1/Major Task 2: Identify alterations in the quantity and distribution of cellular populations and biomolecules that distinguish between PC tumor grades.

Subtask 1: Identify and review existing tissue microarrays containing a range of tumor grades for suitability. Ensure availability so work can commence once approved by the HRPO.

Subtask 1 was completed in month 3, ahead of the scheduled month 4 goal.

Subtask 2: Validate methods for application of mass spectrometry imaging to tissue microarrays (TMA) for prostate cancer (PC).

The studies for Subtask 2 are 80% completed and we anticipate completion by June 15, 2021.

Subtask 3: Validate antigen retrieval, labeling and titrations for imaging mass cytometry of tissue microarrays (TMA) for prostate cancer.

The studies for Subtask 3 were scheduled for completion by month 8 (February 2021); however, due to delays resulting from lab occupancy restrictions during the peak of the COVID19 pandemic we now anticipate completion by month 14 (July 2021).

Subtask 4: Apply validated methodologies to conduct imaging mass cytometry from PC TMA containing a range of tumor grades.

Work for Subtask 4 was slated for completion by month 14 (August 2021). As a consequence of delays in Subtask 3 as described above, we now anticipate completion by month 17 (November 2021).

Subtask 5: Apply validated methodologies to conduct mass spectroscopy imaging of PC TMA containing a range of tumor grades.

Work for Subtask 5 is scheduled for completion by month 14 (August 2021), work is ongoing, and we do not anticipate any delays in meeting this goal.

- **What was accomplished under these goals?**

Major Task 1: Obtain USAMRMC HRPO approval for use of deidentified prostate biopsy samples

During this study period Major Task 1 and the subtasks related HRPO approval were accomplished. The required IRB documentation, CITI approval, and other information was submitted for HRPO approval. Subsequent follow-up questions were addressed, and approval obtained on June 12, 2020. Documentation for continuing HRPO approval for project year two was submitted and notice of receipt and confirmation provided on March 3, 2021.

Specific Aim 1/Major Task 2: Identify alterations in the quantity and distribution of cellular populations and biomolecules that distinguish between PC tumor grades.

Subtask 1: Identify and review existing tissue microarrays containing a range of tumor grades for suitability. Ensure availability so work can commence once approved by the HRPO.

For Subtask 1 Dr. Reeves and Dr. Wu worked together to identify a tissue microarray that contained 84 prostate biopsy cores and one renal tissue sample as a control for prostate specific proteins (Figure 1). The biopsy cores represent a diverse patient cohort representing a range of tumor grades sufficient to meet the scientific objectives. The TMA was generated using 1mm diameter needle biopsies from prostate cancer patients, whose tissue samples were part of the Massachusetts General Hospital Genitourinary Tissue Bank, of which Dr. Wu is the director. The biopsy cores formalin fixed and paraffin embedded (FFPE) in a block in a grid arrangement. The grid layout randomly distributed samples by grade score to avoid potential bias in analysis. The structure of the grid includes strategic gaps to ensure consistent orientation. As part of ongoing work, sections from this

TMA are currently being processed and analyzed by mass spectroscopy imaging to meet the method development goals of Subtask 3 and will be used to complete the scientific objectives of subtask 5.

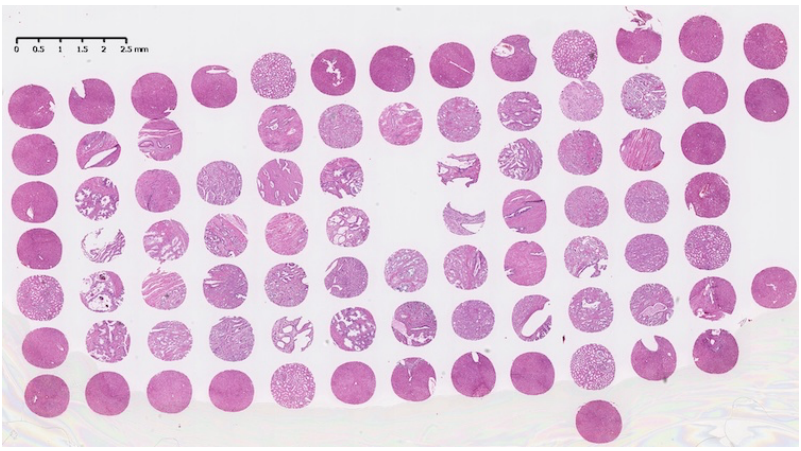

Figure 1. Prostate cancer biopsy tissue microarray. A tissue microarray containing 84 prostate cancer biopsy samples and a kidney tissue control sample was constructed using archival tissues from the genitourinary tissue repository directed by project Co-I Dr. Wu.

Subtask 2: Validate methods for application of mass spectrometry imaging to tissue microarrays (TMA) for prostate cancer (PC).

This subtask first aims to conduct method development for mass spectrometry imaging of the TMA and subsequently generate pilot data, which can then inform the conduct of a validation study. Serial sections of the of the TMA were cut, alternately placing sections onto either Superfrost Gold glass slides or onto slides coated with indium-titanium oxide (ITO) (Table 1). The sectioning strategy used ensures that each slide has an adjacent section above and below that can be imaged using the complimentary methodology, reducing the risk that imperfections or acquisition errors undermine the overall experiment. The Superfrost Gold slides are used routinely for tissue staining, immunohistochemistry, and mass cytometry imaging applications. The sections placed on these slides are dedicated to Subtasks 3 and 4 below. The ITO coated slides provide a conductive surface required for mass spectroscopy imaging while still allowing for light microscopy methods (i.e., H&E staining). After placing sections onto the slides, samples are subjected to antigen retrieval, solvent washes, and enzymatic digest. The samples are then coated with a matrix that absorbs laser energy to liberate analytes from the samples. The details of the antigen retrieval, enzymatic digestion, solvent washes, and matrix coating are important parameters in optimizing the detection of analytes from the samples by mass spectroscopy imaging. To accomplish the optimization and conduct the subsequent imaging of the prostate TMA, we are working with a fee for service provider. Through this arrangement we work collaboratively on the study design and method optimization details and obtain access to a suite of required instruments for the preparation and imaging of samples.

Section #	Slide Type	Section Thickness (mm)
1	Super Frost	5
2	ITO	5
3	Super Frost	5
4	ITO	5
5	Super Frost	5
6	ITO	5
7	Super Frost	5
8	ITO	5
9	Super Frost	5
10	ITO	10
11	Super Frost	5
12	ITO	10
13	Super Frost	5

Table 1. TMA sectioning strategy Serial sectioning strategy for sections of the 84-sample prostate biopsy TMA prepared for imaging mass cytometry and mass spectroscopy imaging.

In anticipation of work for this project, a pilot assessment of a smaller 24 sample prostate tissue TMA was conducted. This pilot study of 24 prostate biopsy cores, 12 of which were cancerous and 12 were from healthy

prostate tissues, evaluated conditions for sample preparation and subsequent imaging by matrix assisted laser desorption and ionization mass spectrometry imaging (MALDI-MSI) methodology, and a resulting set of images were provided (Figure 2). The biopsy cores were imaged at 60µM resolution. Analysis of the resulting images identified several m/z peaks that are enriched in either healthy or cancerous tissues. The data shown in the figure show the distribution of a peak with a m/z of 1642.713 Da, which is enriched in healthy tissues compared to prostate cancer tissue. It was noted that the perimeters of all biopsy cores exhibit elevated levels of this analyte, which likely results from edge effects associated with sectioning and matrix deposition (and is observed for numerous other analytes – not shown). Therefore, the perimeter of the images was not considered for analysis. Based on learning from these initial results and mindful of the objective to coordinate data from both imaging methods, as was anticipated in the proposed work we observed that imaging with higher spatial resolution may be beneficial.

Imaging with higher spatial resolution will likely aid in the integration of data from MSI with imaging mass cytometry, which has a spatial resolution of 1µM. Therefore, a second methodology, Fourier-transform ion cyclotron resonance (FTICR) mass spectrometry imaging, which can provide 20-30µM resolution was suggested for evaluation. Currently several digestion times and matrix compositions are being tested to identify the optimal conditions (Table 2). Unfortunately, due to restrictions and work stoppages resulting from the COVID19 pandemic, completion of these studies was delayed in completion by 2 months. All ongoing work utilizes the larger 84-core prostate TMA shown in Figure 1. These studies are now 80% completed and we anticipate completion by June 15, 2021. The learning points to date and efforts towards development a robust data analysis framework (discussed below) will accelerate the pace of work in the second study period.

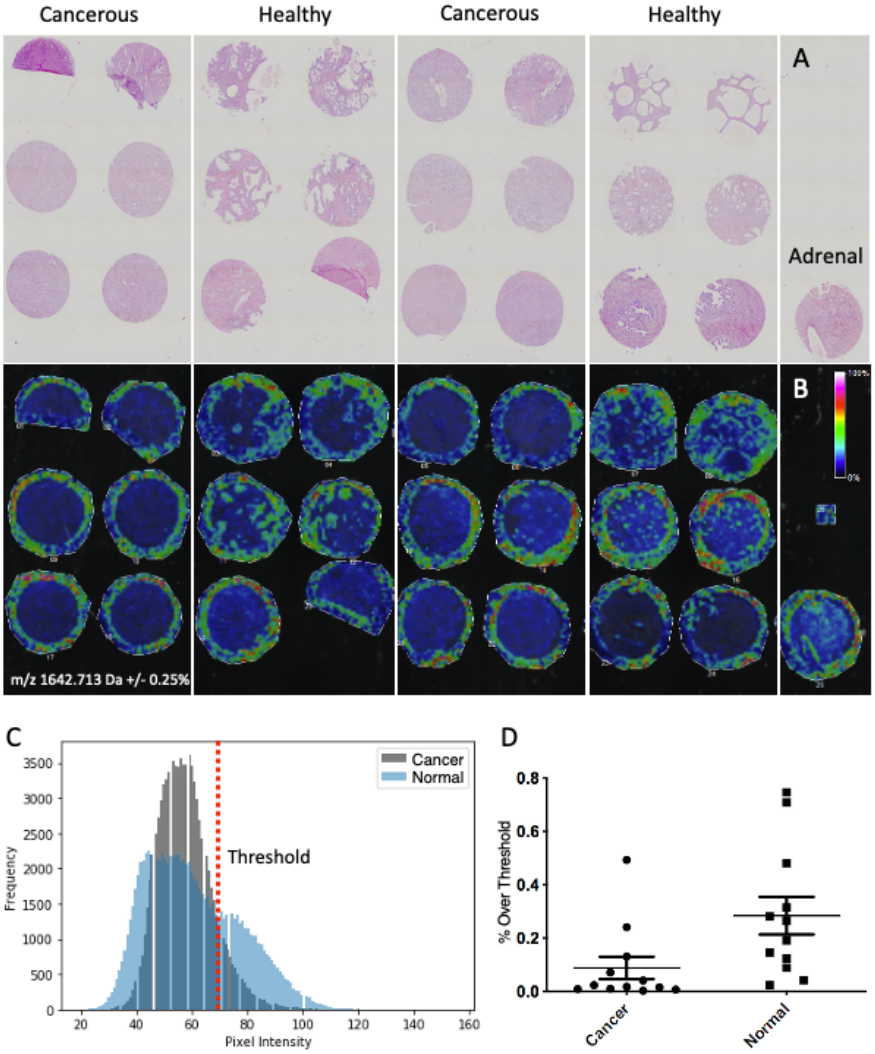

Figure 2. Mass spectroscopy imaging of prostate cancer biopsy tissue microarray. A tissue microarray of 24 tissue biopsy samples and an adrenal tissue control sample was imaged by **(A)** staining with hematoxylin and eosin or **(B)** MALDI-TOF mass spectroscopy imaging (MSI). The peak profiles were analyzed to identify peaks enriched in either healthy or cancerous tissue. An image pseudocolored for abundance of the peak for m/z 1642.713 is shown. **(C)** Analysis of the distribution of signal intensities by pixel from MSI imaging between healthy and cancerous tissues is shown. **(D)** Distribution of percent of pixels above threshold in each image, comparing healthy and cancerous tissue samples.

Subtask 3: Validate antigen retrieval, labeling and titrations for imaging mass cytometry of tissue microarrays (TMA) for prostate cancer.

To assess labeling, imaging, and data processing my imaging mass cytometry, FFPE prostate tissue was sectioned and subjected to heat-based antigen retrieval. Briefly, samples are de-waxed by a wash in xylene and hydrated in ethanol, incubated in heated pH 9 antigen retrieval buffer, and blocked by incubation in BSA solution. Tissues are then labeled with antibody solution and DNA intercalator, washed, and allowed to air dry prior to imaging.

Digestion at 37°C	Matrix
2 hrs / 4 hrs	CHCA
2 hrs / 4 hrs	CHCA
2 hrs / 4 hrs	DHB
2 hrs / 4 hrs	DHB
2 hrs	DHB Ammonium Sulfate
2 hrs	DHB Ammonium Sulfate

Table 2. Sample Preparation conditions for mass spectroscopy imaging. Ongoing optimization work is testing a various combinations of digestion incubation times and matrix compositions to identify the best protocol for overall analyte detection and spatial resolution

To date more than 35 antibodies have been tested at various concentrations to confirm functionality and determine optimal concentration (Table 3). After image acquisition, the raw data is processed and images assembled, followed by single-cell segmentation and downstream data analysis. The workflow to generate images for downstream analysis requires implementation of several software programs including ilastik, cell profiler, and histoCAT. Images from prostate tissue sections resulting from this process demonstrate successful labeling, imaging, and data processing (Figure 3).

Based on results to date and discussions with Dr. Wu, several antibodies either require further optimization or are candidates for inclusion (e.g., PTEN clone HI30, p63, p53 clone DO-7, ERG, AMACR). Studies for Subtask 3 were scheduled for completion by month 8 (February 2021); however, due to delays resulting from COVID19 we now anticipated completion by month 14 (July 2021). However, due to COVID19 pandemic related restrictions access to the imaging mass cytometer instrument was severely restricted. Therefore, refinements to the antibody panel including the addition of newly identified antibodies of interest and final validation of antibody labeling of the prostate cancer tissue microarray antibody is not yet completed. The work to finalize and validate the antibody panel is ongoing and we anticipate completion by end of July 2021.

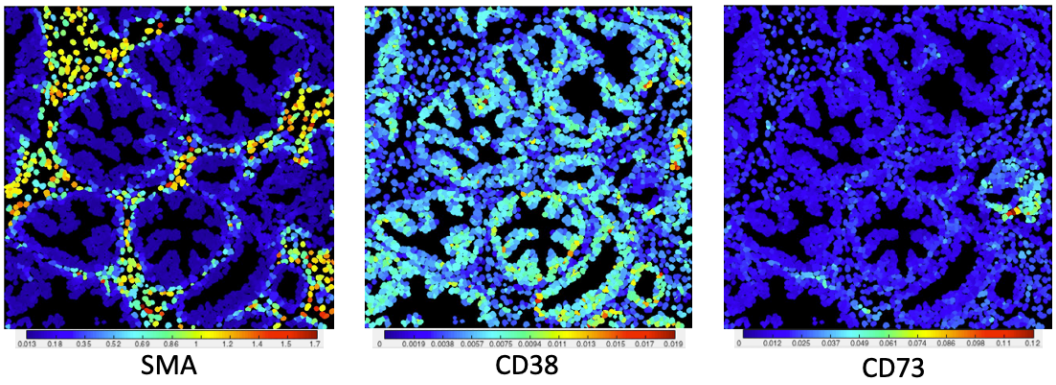

Figure 3. Imaging mass cytometry of prostate tissue sections. Three panels depicting the different proteins in same prostate tissue section. The images were generated by image processing, single cell segmentation, finally pseudocoloring. Across all images labeled nuclei of individual cells are represented in blue. Indicated proteins, SMA, CD38, or CD73, are colored by intensity (black low, red high).

Subtask 4: Apply validated methodologies to conduct imaging mass cytometry from PC TMA containing a range of tumor grades.

Application of the validated methodologies from subtask 3 was scheduled to initiate in month 8 of the project and conclude by month 17. However, final validation of the antibody panel has not been achieved as a result of delays related to COVID19 lab occupancy restrictions. Consequently, we anticipate that Subtask 4 will be completed by month 17 (November 2021) of the study rather than the initially estimated month 14 (August 2021).

Subtask 5: Apply validated methodologies to conduct mass spectroscopy imaging of PC TMA containing a range of tumor grades.

The overall experimental plan and tissue sample preparation for execution of Subtask 5 are now completed. The data for clinical annotation of the samples has been updated in conjunction with Dr. Wu. The results from remaining work for Subtask 2 will inform final decisions for technical parameters for the imaging of the 84 core TMA described in Subtask 1 (above). The schedule for this study anticipates completion by month 14 (August 2021), at the current pace of work we do not foresee problems with meeting this timeline.

Other achievements

Due to COVID19 restrictions on lab occupancy, progress on several aspects of experimental and technical goals was reduced for some aspects of the first report period. Consequently, an additional effort was placed on the planning and development of a data analysis workflow to process and integrate data from both imaging methods, a fundamental component underpinning work throughout the study. The rationale was that development of the workflow using available data will maintain productivity and introduce efficiencies to accelerate work in the second and third study periods. Data analysis workflows exist for the analysis of imaging mass cytometry data as well as for mass spectroscopy imaging data. However, to date no tools are publicly available that can integrate data from two high-dimensional imaging methods. Integrating or layering data together from both methods into one cohesive image will enable analysis of tissue sections with an exceptional amount of detail while also presenting several technical challenges including limiting computational burden, image registration, data alignment and downstream analysis.

To meet these challenges and drive the analysis of data resulting from this project, we are developing a workflow to align and register data from multiple high-dimensional imaging methods that allows transfer of information between images to explore cell phenotypes and microenvironment features using fully linked data from both imaging methods while including quantifiable performance metrics (Figure 4). Importantly, this workflow aims to optimize performance while reducing computational burden and does not incorporate deep learning strategies. Deep learning requires large data sets for training and

Antibody Target	Metal Label
CD38	141Pr
SMA	141Pr
EGFR	142Nd
Vimentin	143Nd
CD14	144Nd
EpCAM	144Nd
CD33	145Nd
CD16	146Nd
CD163	147Sm
ICOS	148Nd
CCR4	149Sm
PD-L1	150Nd
OX40	151Eu
CD45	152Sm
Lag3	153Eu
CD11c	154Sm
FoxP3	155Gd
CD4	156Gd
E Cadherin	158Gd
CD68	159Tb
VISTA	160Gd
CD20	161Dy
CD8a	162Dy
CCR6	163Dy
Arginase-1	164 Dy
b-Catenin	165 Ho
B7-H4	166Er
Granzyme B	167Er
Ki-67	168 Er
Collagen I	169Tm
CD3	170Er
Histone H3	171Yb
Caspase3	172Yb
CD184/CXCR4	173Yb
CD45RO	173Yb
HLA-DR	174Yb
Pan-Actin	175 Lu
c-Myc	176Yb

Table 3. Tested Antibodies Metal conjugated antibodies previously valuated for labeling tissues for IMC

validation, and currently there are no models available for alignment of multiple high-parameter images. Additionally, the results of deep learning tools are often "black boxed" and cannot be explained or explore in a stepwise manner, while our approach is modular, and results can be interrogated at each step.

To provide a reference images and capture overall tissue morphology, including any deformations resulting from processing, our approach takes advantage of the fact that mass spectroscopy imaging is compatible with H&E staining to provide tissue architecture, while imaging mass cytometry data can be used to create pseudo-H&E by combining DNA and collagen signals (Figure 5). These H&E images can be used to identify landmarks and overall tissue features to provide guides for image alignment.

The high dimensionality of the data would require manual alignment of >1000 individual parameters for each tissue imaged, resulting in a workflow that is not feasible. Identifying image features and aligning data computationally is much more efficient; however, doing so for each image would be exceptionally resource intensive if all the parameters of both imaging methods were used. One strategy to combat the computational cost is to use dimension reduction tools such as principal component analysis (PCA), tSNE, UMAP, which decrease high-dimensional data to limited number of parameters while preserving the relationships between data points from the original information. To date we have evaluated several dimension reduction tools identify the algorithm that balances computational runtime and data fidelity using a representative prostate tumor image data set (Figure 6). The results from these tests indicate that nonlinear methods such as t-SNE and Isomap require longer run times than the nonlinear methods PHATE and UMAP. Linear methods, such as PCA and NMF, have the lowest run times; however, they do not capture data complexity efficiently and require a higher number of dimensions. When evaluating the preservation of the data structure, or fidelity, using the Spearman correlation of geodesic and Euclidean distances in the data (denoised manifold preservation – DeMAP) the nonlinear methods Isomap, PHATE, and UMAP all

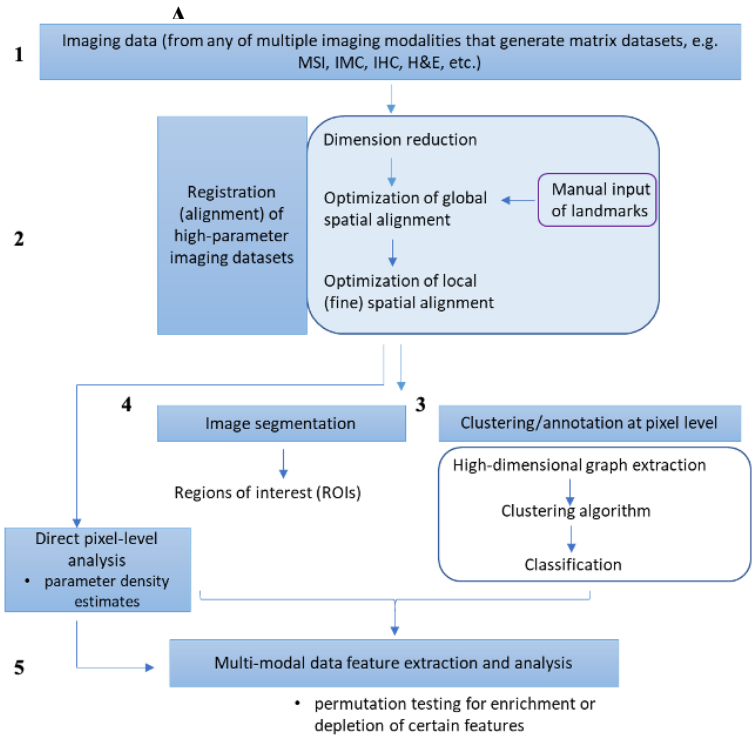

Figure 4. Workflow diagram for integration and analysis of multiple high-dimensional imaging methods.

1 Data imaging methods, high or low dimensional, are generated and preprocessed. **2** Dimension reduction of each input image (e.g., PCA or tSNE) to enable efficient image registration of imaging data. **3** Cluster data (e.g., manifold, k-means, fuzzy simplical) and classify to identify landmarks **4** Segment images by threshold masks and remove artifacts to assign data to single cells **5** Multi-image data is merged and can be analyzed by computational and statistical tools

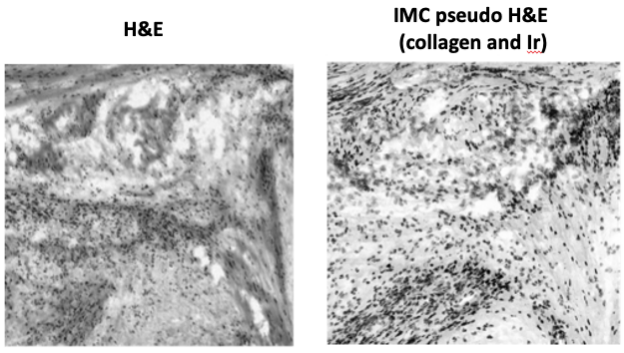

Figure 5. Comparison of staining by H&E and pseudo-H&E from imaging mass cytometry. Adjacent sections of the tissue were either stained for H&E or labeled for IMC and imaged. The pseudo-H&E is generated by combining signal for antibody labeled collagen and DNA (Ir – iridium). Consistent features in the tissue architecture are identified and support the integrated mapping of data from mass spectroscopy imaging and imaging mass cytometry.

consistently preserve manifold structure without prior data filtering of the data while achieving correlations above 0.85 across dimensions 2-10.

Once processed, dimension reduced datasets can be registered to the corresponding H&E image. As part of ongoing work, we are currently testing methods to quantify and evaluate the quality of the image registration. Once we have validated the image registration approach, data from both imaging methods integrated in one cohesive structure that preserves and aligns the spatial information, creating a highly detailed map of each tissue biopsy sample.

These integrated datasets can then be applied to already available tools for image segmentation (ilastik), spatial relationship analysis (histoCAT), statistical testing (edgeR, correspondence analysis), and machine learning tools. Together these analytical tools can quantify the phenotypes of various cell populations, identify distributions of cell populations and biomolecules, and determine how the spatial relationships between these features correspond to clinical data. We anticipate submission of a manuscript describing the development and validation of this method in detail by the end of July 2021.

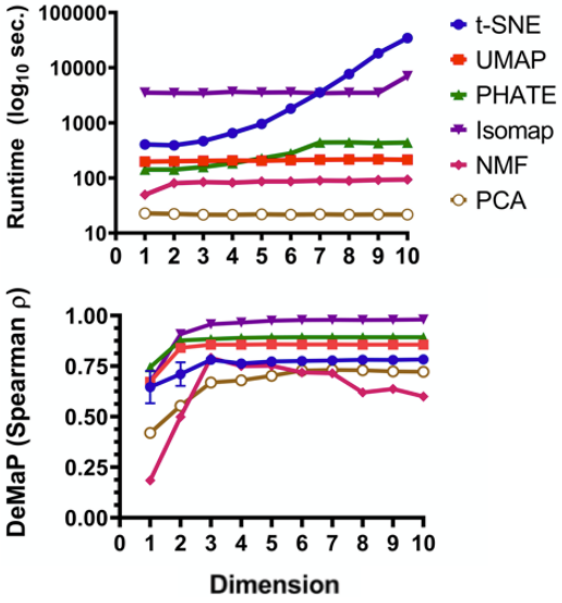

Figure 6. Evaluation of speed and fidelity for multiple dimension reduction methods. **A** Computational runtime for each algorithm across embedding dimensions 1-10. Plotted are the mean and standard deviation (n=5) across each number of dimensions for each method. **B** Denoised manifold preservation (DeMaP) metric between Euclidean distances in resulting embeddings corresponding to non-peak-picked data and geodesic distances in ambient space (not dimension reduced after peak-picking) of corresponding peak-picked data. Results showing the mean and standard deviation DeMaP metric (Spearman's rho correlation coefficient) for all tested dimension reduction methods (n=5).

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

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

The first two objectives are to close the gap in the execution timeline by completing the Subtasks 3 and 4 from Year 1 Major Task 2 to generate imaging mass cytometry data and ensure that the generation of the mass spectroscopy imaging data remains on schedule.

In addition, the final subtask for year 1 is to prepare a manuscript on the application of the layered imaging methodology to tissue samples. As described above, work to develop a data analysis workflow to integrate high-dimensional images and conduct downstream analysis using computational and statistical tools initiated during

year one in response to constraints from the COVID19 pandemic. We anticipate that a manuscript describing the analysis methodology, underlying mathematical and computational approaches, and proof of concept data analysis demonstrations will be submitted by July 2021. A second manuscript focused on the detailed phenotyping of cell populations, investigation into the tumor microenvironment and identification of image analysis derived correlates to prostate biopsy grade will follow.

We anticipate that the year 1 mass spectroscopy imaging will be completed on schedule. Analysis and refinements from the execution of year 1 studies will inform any refinements for the methodology applied to the second year TMA. Currently, we anticipate only minor adjustments to the methodology for mass spectroscopy imaging as applied to Year 2 studies.

Dr. Reeves and his lab will focus on the final experiments and data analysis for the first prostate TMA to conclude those studies and begin data analysis from both imaging methods by November 2021. As data analysis and manuscript preparation for the year 1 TMA is ongoing, we will work with Dr. Wu to initiate the construction of a second TMA to meet the goals of Major Task 3, the first Year 2 goal.

The objective of the year 2 study is to identify hallmarks of changes in cell population frequencies, spatial organization and other tissue features that correlate to risk of progression. Considering the current project timeline, we will initiate the process of constructing a TMA ahead the scheduled month 14 to avoid delays and potentially recoup some lost time. Completing the year 1 analysis prior to imaging the Year 2 TMA will allow for insights and improvements to be incorporated from the analysis from the first TMA. In addition, this time will allow for a literature review to tailor the antibody panel to the second-year research objectives.

We anticipate that the optimization and imaging mass cytometry analysis of the TMA for Year 2 objectives will be completed by month 20, a 2-month delay. However, we do not anticipate delays in the execution of the mass spectroscopy imaging experiments for Year 2 and will be completed by month 18. Computational analyses will be completed by month 22, with the goal to conclude Year 2 activities prior to the conclusion of the second study period.

4. **IMPACT:**

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

The work to date will both benefit the specific area of prostate cancer research as well as have broader applications to other areas of cancer research, imaging, and computational analysis.

The optimized methods for tissue preparation, including antigen retrieval, antibody labeling, enzymatic digestion, and matrix coating will aid future studies conducted as part of this project as well as work from other groups that may pursue similar research strategies. Similarly, details regarding methods to facilitate image acquisition by imaging mass cytometry and mass spectroscopy imaging will also be of value to the research community.

A major hurdle in the use of multi-modal imaging is the co-registration of images and data from each independent method. A significant portion of work during the first period focused on developing computational strategies that efficiently and robustly align high-dimensional imaging data. The analytical workflow currently under development enables the integration of multiple imaging methods to create one composite high-dimensional image. In addition, the analytical tools can be used to train predictive algorithms that can use one imaging method to reliably predict results from a second imaging method. The ability to make predictive associations is of particular value when not all imaging methods are not feasible to execute due to limitations in

sample or instrumentation. The ongoing development of the workflow will also enable correlation of image features, groups of features, and ratios between features to clinical data. These correlations will be used to identify those components from imaging that are strongly linked to cancer grade scores and identify potential prognostic markers.

We anticipate that in the next project period a manuscript detailing the methods and computational tools developed will be prepared and submitted for publication. A second manuscript will specifically address the findings regarding the detailed analysis of prostate cancer biopsy and identification of features to enhance the discrimination of prostate cancer grade groups. Reporting of these findings will be of value to researchers that aim to improve their understanding of the biology of prostate cancer, and other cancers, or improve on prognostic tests to inform clinical practice.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. **CHANGES/PROBLEMS:**

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

The most significant problems and delays experienced during the report period are a direct result of the ongoing COVID19 pandemic. The research and clinical activities within Massachusetts General Hospital, the study site, were significantly restricted. Following a brief period that included a full prohibition of non-COVID19 or otherwise essential research activities, research activities were limited to 50% occupancy, with most core facilities closed or operating at reduced capacity. Importantly, access to both the imaging mass cytometer and mass spectroscopy imager was limited and created several challenges in conducting experiments. Similarly, supply chain constraints for laboratory consumables, antibodies, and other reagents also resulting from COVID19 have also impacted execution of studies.

Several steps have been or are being taken to resolve these challenges. Normal work activities have largely resumed, with few restrictions that do not impact the capacity to conduct studies. Second, we have adapted to identify alternative vendors and build in larger lead times when needed to procure necessary reagents and laboratory materials. Third, a previous constraint in accessing instrumentation for imaging mass cytometry will soon be resolved. Previously, accessing the instrument for imaging mass cytometry required travel to Yale University. The scheduling and logistical constraints combined with COVID19 restrictions significantly impaired access to the device. However, an imaging mass cytometer instrument will be available locally within Boston by July 2021 and will significantly improve access, scheduling, and execution of imaging studies. Finally, development of the data analysis workflow during year one will introduce efficiencies the analysis workflow will accelerate pace of analysis and reporting to help alleviate the current time constraints.

Changes that had a significant impact on expenditures

Though no overall change in planned studies, personnel, or other charges occurred, delays resulting from the COVID19 pandemic reduced expenditures during the first year. Charges for mass spectroscopy imaging services should have been incurred for the first year and while first year tasks are nearing completion and the funds have been allocated internally, the vendor has not yet submitted an invoice. Similarly, charges for reagents and run time for use of the imaging mass cytometer were delayed. Provided that remaining fund from the first year are carried over, the remaining funds will be sufficient for completing work for year 1 tasks. We do not foresee these challenges continuing during the second study period and anticipate study activities and expenditures.

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

Nothing to report.

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.**

  Nothing to report.

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

  Nothing to report.

- **Technologies or techniques**

  Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

  Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Patrick Reeves, PhD*
Project Role:	*Principal Investigator*
Researcher Identifier:	*ORCID 0000-0001-8202-0723*
Nearest person month worked:	4 months
Contribution to Project:	*Coordinated and execute administrative tasks including IRB and HRPO approvals for samples, reagent and vendor purchases, collaborator, and team member efforts. Research efforts included experimental and antibody panel design, assay evaluation, development of analysis workflows, and generation of draft manuscript for data analysis methodology.*
Funding Support:	

Name:	*Chin-Lee Wu, MD PhD*
Project Role:	*Co-investigator*
Researcher Identifier:	*ORCID 0000-0002-0444-0068*
Nearest person month worked:	*0.6 months*
Contribution to Project:	*Worked on project idea development, study design including antibody selection, and prostate cancer sample selection*
Funding Support:	

Name:	*Mark C. Poznansky, MD PhD*
Project Role:	*Co-investigator*
Researcher Identifier:	*ORCID 0000-0003-1344-7103*
Nearest person month worked:	*0.6 months*
Contribution to Project:	*Advised on project coordination, study management, as well as clinical data and sample selection.*

Funding Support:	

Name:	*Ann Sluder, PhD*
Project Role:	*Project manager*
Researcher Identifier:	*ORCID 0000-0003-4415-0652*
Nearest person month worked:	*0.6 months*
Contribution to Project:	*Supported directly through project management, administrative and financial tracking. Advised on project coordination and vendor discussions.*
Funding Support:	

Name:	*Josh Hess*
Project Role:	*Technician*
Researcher Identifier:	
Nearest person month worked:	*4 months*
Contribution to Project:	*Development of analysis of computational data analysis tools and design of analysis workflow. Generation of draft manuscript related to data analysis methodology.*
Funding Support:	

Name:	*Shulin Wu, MD PhD*
Project Role:	*Research Fellow*
Researcher Identifier:	
Nearest person month worked:	*3 months*
Contribution to Project:	*Worked on selection and preparation prostate biopsy sample, image collection, data collection and annotation.*
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

One project for which Dr. Reeves committed effort concluded during the report period. Specifically, the completed project, a sponsored research agreement R42AI120269-03 "Restimulating memory T cell responses in elderly by a novel, live influenza vaccine", had been allocated 20% effort. This change did not impact Dr. Reeves' effort on this project, W81XWH2010301 Identification of novel features to assess risk and improve

therapeutic decision making for prostate cancer, during the report period nor will it impact work in the next research period.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Not applicable.

9. APPENDICES:

Not applicable.