Nonlinear Microscopy Provides Non-destructive Rapid Pathology Annotations for High-quality Prostatic Tissue Procurement and Translational Research

Timothy D. Weber[1], Roshan Dsouza[1], Lucas C. Cahill[1], Tadayuki Yoshitake[1], Andrew A. Wagner[2], Peter Chang[2], Boris Gershman[2], Seymour Rosen[3], James G. Fujimoto[1], Huihui Ye[4], Yue Sun[3]

Affiliations
[1]Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology
[2]Department of Urology, Beth Israel Deaconess Medical Center, Harvard Medical School
[3]Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School
[4]Department of Pathology, Cedars-Sinai Medical Center

Background
Fresh tissue collection with accurate pathology annotations remains a significant bottleneck in achieving high-quality translational oncological research. Prostate cancers are often macroscopically unrecognizable and exhibit considerable inter- and Intra-tumoral heterogeneity in tumor grades and immune environment. Existing methods for fresh prostate tissue collection have significant limitations that can negatively impact tissue integrity and cell viability. Nonlinear microscopy (NLM) technology, a novel optical sectioning technology, offers rapid visualization of microscopic histomorphology in fresh tissue within minutes. This novel method avoids the need for processing and microtoming, enabling collection of high-quality fresh prostatic tissue for research. In this study, we aimed to evaluate the accuracy of NLM in pathology annotations and its potential impact on tissue integrity for downstream experiments.

Materials and Methods
We performed NLM analyses on a series of 155 fresh core biopsies from 53 men and 122 fresh tissue samples procured from 40 men undergoing radical prostatectomies. Fresh prostatic tissues were stained for nuclear and cytoplasmic contrast with acridine orange (AO) and sulforhodamine 101 (SR101) in a 50% ethanol solution for two minutes, followed by a 30-second saline rinse. Tissues were then imaged in real-time using NLM generating digital images in an H&E color scale and compared with standard H&E-stained, paraffin-embedded histology (Figure 1). NLM images and corresponding H&E stains were evaluated by 3 pathologists independently and double-blinded. Tumors enriched for tumor-infiltrating lymphocytes (TILs) and benign control tissues were identified using NLM. Lymphocytes were isolated and subjected to flow cytometry analyses (Figure 2).

Results
NLM demonstrated a sensitivity of 92.4% and 97.3%, and a specificity of 100% and 100%, for detecting carcinoma in fresh biopsy cores and fresh tissue procured from prostatectomies, respectively. NLM's ability to image up to 100 μm below the tissue surface allowed for three-dimensional visualization, effectively resolving ambiguities in Gleason patterns that might arise from single-depth sections. NLM did not interfere with subsequent H&E histology, flow cytometry, immunohistochemistry, and molecular testing.

Conclusions
NLM enables rapid, non-destructive imaging, offering real-time diagnostic support and potential integration with digital pathology and AI algorithms to enhance tissue procurement and translational research. This technology provides rapid assessment biopsy and surgical tissue, all while maintaining tissue integrity for subsequent research. Evaluation of post-NLM tissue viability for tissue cultures and its compatibility with xenografts, organoids, and GEMM models, will be performed in the immediate future.

Funding Acknowledgements: NIH R01 CA249151
Conflicts of Interest Disclosure Statement: LC, TY, JF are inventors of unlicensed, intellectual property on NLM owned by MIT. The other authors have no conflicts of interest related to the content of this abstract.

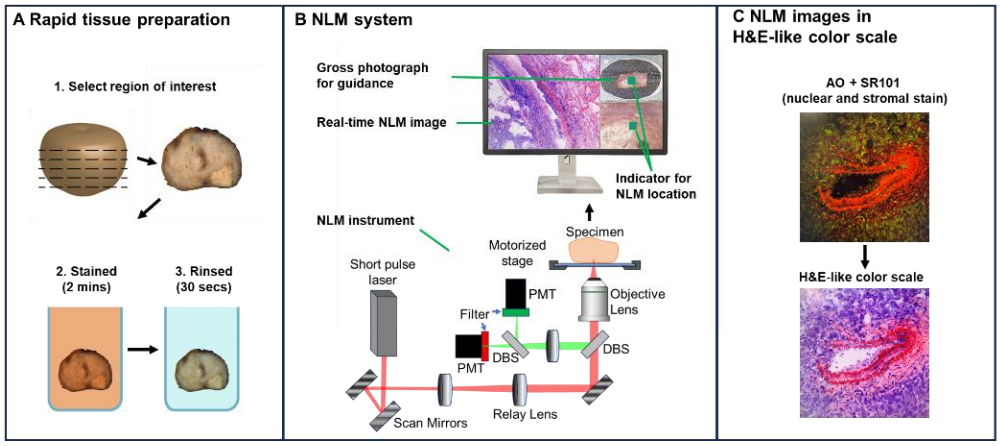

Figure 1. Tissue processing and NLM imaging. A. Tissue sample was stained in a solution of AO & SR101, followed by saline rinse. B. The specimen was then placed on the glass window of a specimen holder in an NLM instrument, translated in the horizontal (x-y) plane to the region of interest, and the microscope's focus depth, vertical (z-axis) translation, could be adjusted up to 100 µm below the surface. A position indicator was displayed on a white-light image of the gross specimen to guide NLM image positioning. The specimen holder allows imaging of specimens measuring up to 7 x 10 cm. C. The NLM images were displayed in a H&E-like color scale at 16 frames per second.

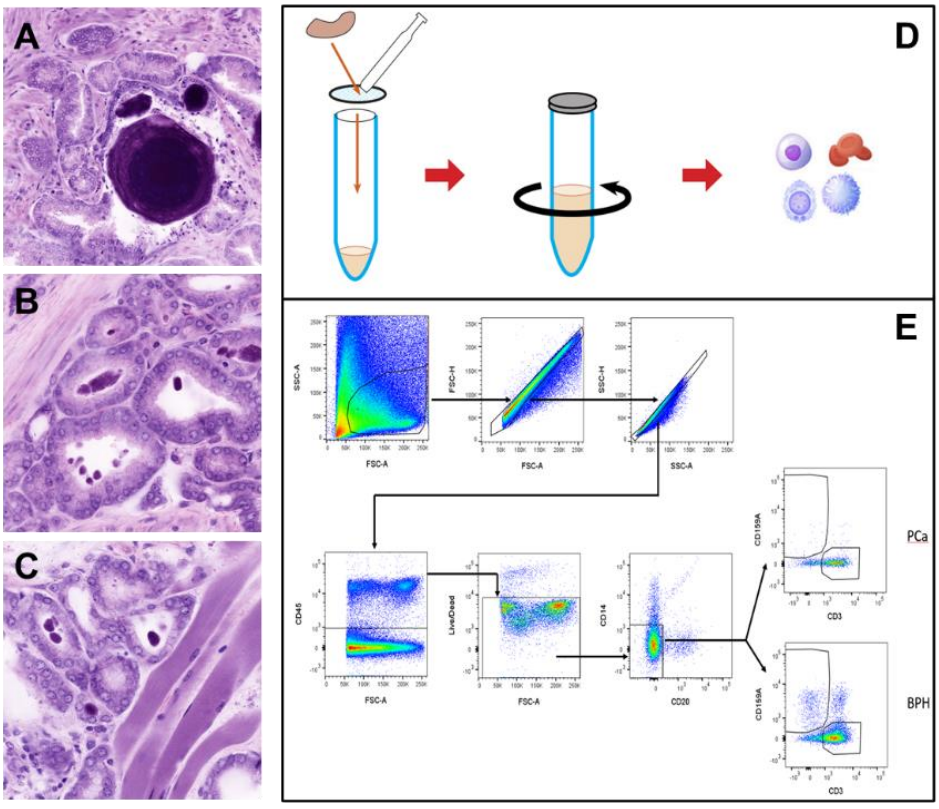

Figure 2. Representative NLM images of prostate pathology and an example of TIL isolation and phenotyping using NLM-guided tissue procurement. A. A representative image of benign glands with corpora amylacea. B-C. Representative images of Gleason pattern 3 cancer with luminal secretions and adjacent skeletal muscle. D. A schematic illustration of fresh prostate tissue procurement and TIL isolations. TIL-enriched tumor and benign tissue were identified using NLM, mechanically disrupted, and filtered through a nylon mesh screen. Single cell suspensions were centrifuged at 500 x g for 5 min and the cell pellet was washed before staining. E. Collected single cell suspensions were stained, sorted, and subjected to flow cytometry assays.