Non-destructive 3D pathology with open-top light-sheet microscopy

Nicholas P Reder*, Adam K Glaser**, Weisi Xu**, Lindsey Barner**, Jonathan TC Liu**, and Lawrence D True*

Departments of Pathology* and Mechanical Engineering**, University of Washington, Seattle, WA

Background: Glass slide-based pathology has been the gold standard for prostate cancer diagnosis for many decades. However, this process is resource-intensive, decreases nucleic acid yield for molecular assays, and diagnostically useful 3D information is lost. We have designed a protocol which produces a 3D digital microscopic image of entire prostate biopsy cores while preserving nucleic acids for downstream testing. We expect that our technology will improve prostate cancer diagnostic precision, enhance molecular diagnostics, and lead to new biologic insights with 3D structural information.

Methods: Ex-vivo core needle biopsies taken from radical prostatectomies were dehydrated, chemically clarified to render the tissue transparent, and stained with nuclear (TO-PRO3) and cytoplasmic (eosin) fluorescent dyes. An ethanol-based nucleic acid preservative was used to fix and store the tissue. 3D immunofluorescent staining for CK8 was performed using a week-long incubation protocol. Biopsies were imaged using a custom-built open-top light-sheet microscope (OTLS). The images were reconstructed and false-colored to simulate H&E-staining. Custom Python scripts and BigStitcher were used for image processing, and Imaris software for 3D visualization.

Results: Biopsies were entirely imaged in 3D within 20 minutes after clarification (~6-12 hours). Pseudo-H&E staining showed the benefits of 3D microscopy, including avoidance of overgrading ambiguous regions (well-formed glands vs. poorly-formed glands), and the presence of higher grade patterns that would have otherwise been missed with 2D slide-based pathology. There were rare cases of biopsies with carcinoma detected in 3D that would have been classified as benign with 2D slide-based pathology.

Conclusion: Examination of prostate cancer 3D histoarchitecture yields more precise grading than conventional grading of 2D sections. Most importantly, 3D microscopy detected cases of overgrading, undergrading, and missed carcinoma. This finding has major implications for patient care, particularly the decision to remain in active surveillance versus being offered intent-to-cure therapy. Further work to characterize novel 3D cancer morphology and multiplex with additional molecular biomarkers is in progress.

Conflicts of interest: The authors (NPR, AKG, JTCL, and LDT) have a patent application for the open-top light-sheet microscope and have equity in a start-up company (Lightspeed Microscopy, Inc) commercializing the technology. Dr. Reder is the CEO of Lightspeed Microscopy.

Funding Acknowledgements: PCF Young Investigator Award, DOD PC170176, DOD PC180686