**3D light-sheet microscopy for next generation prostate pathology**

Nicholas P Reder*, Adam K Glaser**, Erin McCarty*, Ye Chen**, Jeffrey Chia**, Chengbo Yin**, Peter Wei**, Jonathan TC Liu**, and Lawrence D True*

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

**Background:** Basing the diagnosis of prostate cancer on hematoxylin and eosin (H&E)-stained sections from clinical specimens has been the gold standard for many decades. However, this process is resource-intensive, decreases nucleic acid yield for molecular assays, and results in sampling errors. Furthermore, diagnostically useful 3D information is lost. For example, inter-pathologist agreement for distinguishing Gleason pattern 3 glands from poorly-formed Gleason pattern 4 glands is low (kappa = 0.3). Making that distinction categorizes patients for active surveillance (pattern 3) vs. intent-to-cure therapy (pattern 4). We have designed a protocol which produces a 3D digital microscopic image of entire prostate biopsy cores. We expect that this protocol will improve prostate cancer diagnostic precision and preserve nucleic acids for downstream testing.

**Methods:** Ex-vivo core needle biopsies taken from radical prostatectomies were chemically clarified, to render the tissue transparent, and stained with nuclear (DRAQ5) and cytoplasmic (eosin) fluorescent dyes. Biopsies were imaged using a custom-built light-sheet microscope. The images were reconstructed and false-colored to simulate H&E-staining. Custom Python scripts were used for image processing, and Imaris for 3D visualization.

**Results:** Biopsies were entirely imaged in 3D within 3 minutes after rapid clarification (<15 minutes). Approximately 400 optical sections, spaced 1 micron apart in the z-axis, were examined for each core. Regions with the poorly formed gland variant of Gleason pattern 4 were proven to be tangential sections of Gleason pattern 3 cancer glands by scrolling through 1 micron optical sections in the z-axis. The RNA integrity number, used to assess the quality of the RNA, was similar to that of samples immersed in RNAlater.

**Conclusion:** Examination of prostate cancer 3D histoarchitecture yields more precise grading than conventional grading of 2D sections. Most importantly, tangentially sectioned Gleason pattern 3 glands can be distinguished from the poorly formed gland variant of Gleason pattern 4. This finding has major implications for patient care, particularly the decision to remain in active surveillance versus being offered intent-to-cure therapy. In addition, our protocol preserves RNA for potential downstream molecular assays to further improve risk stratification. 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) hold a provisional patent for the light-sheet microscope and have a start-up company (Alpenglow Optics, LLC).

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