3D Pathology: Use of Clarity & Light-sheet Microscopy to More Accurately Grade & Stage Unfixed Prostate Carcinoma

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

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

Background The histopathological features of prostate cancer (grade, stage, margin status), which are based on 2D sections of tissue, guide patient management. However, features may be so focal as to not be definitive. For example, inter-pathologist variance distinguishing tangential sections of Gleason pattern 3 glands [the basis for Gleason score 6 cancer] from the poorly-formed gland variant of Gleason pattern 4 glands [the basis for Gleason score 7 cancer] is high (kappa = 0.3; PMID 21679996). That distinction categorizes patients for active surveillance vs. intent-to-cure therapy. Likewise, tiny foci of extracapsular invasion and of cancer abutting vs. involving the margin vary with plane of section (PMID:24525503). Visualizing tissue in 3D may resolve these issues. A light-sheet microscope (LSM) can rapidly visualize tissue in 3D in a slide-free, non-destructive manner, if the natural opacity of tissue (due to lipids) and light refraction is minimized..
Methods Slices of prostatectomy tissue and needle biopsies were clarified to remove lipids (PMID 23575631) and stained with fluorescent dyes (DRAQ5/nuclei, eosin/stroma, and anti-keratins). Refractive index matching solutions minimized light scattering. Our LSM system provided sequential one micron thick digital images of 3 x 3 x 0.4 cm tissue at 2 microns spatial resolution within 15 minutes. MatLab and ImageJ were used for image processing, and Imaris for 3D visualization.
Results Resolution of images of prostatectomy slices was sufficiently high to identify cancer within the timeframe of an intraoperative consultation. Slices without cancer need not be processed for histology, thereby saving cost. Prostate needle biopsies were imaged in 3D within 3 minutes. Approximately 400 optical sections, 1 micron apart in the z-axis, revealed that benign prostate glands form a complex maze throughout the z-axis. In some cores, regions of the poorly formed gland variant of Gleason pattern 4 proved to be tangential sections of Gleason pattern 3 cancer glands by scrolling through sequential 1 micron optical sections.
Conclusions A LSM system rapidly and non-destructively images fresh tissue for intraoperative consultation. The LSM system can decrease costs while maintaining quality care by processing for histology only those tissue slices from prostatectomies which have carcinoma. Finally, we can more comprehensively examine prostate core needle biopsies by clarification and LSM than is done by conventional methods. Current histologic processes examine 2D structures within 5 um thick sections of tissue. Examination of cleared prostate core biopsies in 3D (as 1 micron thick digital sections) adds more accurate diagnostic information than conventional histology. Tangentially sectioned Gleason pattern 3 glands can be distinguished from the poorly-formed gland variant of Gleason pattern 4 glands. Finally, detailed phenotyping of cell clusters in 3D may reveal malignant subclones spatially distributed in prostate cancer in a predictable pattern.

Conflict of Interest None

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