

Prospective External Validation of Stimulated Raman Histology and Machine Learning for Rapid Prostate Cancer Detection and Tumor-Enrichment from MRI-Targeted Biopsies

Takeshi Namekawa, MD, PhD^{1,2}; Lea Lough, PhD²; Adrian Ion-Margineanu, PhD³; Mingyu Sheng, MSc²; Balaji Pandian, MD³; Eric Belanger, MD⁴; Kamal Al Najem Azzam²; Fang-Ming Deng, MD, PhD⁵; Colin Collins, PhD²; Josh Scurll, PhD²; Ewan Gibb, PhD^{2,6}; Amina Zoubeidi, PhD²; Alexander W Wyatt, PhD^{2,6}; Martin E. Gleave, MD^{1,2}; Christian Freudiger, PhD³; Samir S. Taneja, MD⁷; Miles P. Mannas, MD, MS^{1,2}

1. Dept. of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada
2. Vancouver Prostate Centre, Mohseni Institute of Urologic Sciences, Vancouver, BC, Canada
3. Invenio Imaging, Santa Clara, CA, United States
4. Dept. of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada
5. Dept. of Pathology, NYU Langone Health, New York, NY, United States
6. Dept. of Basic and Translational Research, BC Cancer Research Institute, BC Cancer, Vancouver, Canada
7. Dept. of Urology, Northwell Health, New York, NY, United States

ABSTRACT

Background:

Molecular profiling in prostate cancer (PCa) is limited by biopsy specimens with insufficient tumor or degraded nucleic acids. Although magnetic resonance imaging (MRI)-targeted biopsy improves lesion sampling, it provides no real-time confirmation if sampled cores contain adequate tumor or are adequate for sequencing. We prospectively validated a machine learning algorithm for PCa detection with stimulated Raman histology (SRH-AI) and guide selection/trimming of tumor-enriched tissue for molecular profiling.

Methods:

Prospectively, between June 2024–February 2025 we externally validated an SRH-AI for PCa detection with 200 consecutive men with 256 MRI regions of interest undergoing a transperineal MRI targeted prostate biopsy, Table 1. Fresh, unfixed MRI-targeted biopsy cores were imaged on the NIO Laser Imaging System (Invenio Imaging Inc. Santa Clara, CA) using either the dual-channel protocol (CH2+CH3) or a single-channel protocol (CH2-only). Single-channel imaging provides lipid-weighted contrast sufficient for AI-based analysis, whereas dual-channel imaging is required for generation of pseudo-H&E images suitable for human review. SRH images were analyzed using the SRH-AI CNN (Inception-ResNet-v2) incorporated into the NIO to classify biopsy tissue as benign or PCa infiltrated. SRH-AI produced spatial, per-pixel maps of model-predicted tumor area quantification, Figure 1. After SRH imaging, biopsies were placed in liquid nitrogen for tissue biobanking or formalin for routine pathologic processing and ground truth diagnosis, Figure 1. The SRH-AI guided triage and trimming of MRI targeted research biopsy cores before cryopreservation to ensure at $\geq 50\%$ tumor by SRH-AI area, in 163 men consenting to tissue biobanking. Selected frozen cores representing variation in grade, biopsy length, number of trimming iterations, number of SRH scans and cancer core involvement underwent a targeted PCa DNA sequencing panel (compared to formalin fixed biopsy and prostatectomy published cohorts) and whole transcriptome RNA sequencing (compared to fresh frozen published cohort), Table 2.

Results:

The SRH-AI detected PCa with a biopsy-level area under the receiver operating curve of 0.93 in a 2.5-minute-per-core-scan and 0.935 in 5-minutes-per-core-scan. Based on SRH-AI output, biopsies were not

cryobanked (low predicted PCa area, n=135), trimmed before cryobanking (intermediate PCa area; n=45 with n=28 initially <50% PCa predicted area), or cryobanked without trimming (high PCa area; n=20). SRH-AI guided trimming increased median SRH-AI-estimated PCa area from 45% (IQR 40–55%) to 72% (IQR 58–81%) (p<0.001). DNA sequencing succeeded in 17 of 17 selected frozen cores. Compared with published formalin-fixed paraffin-embedded cohorts, selected frozen cores had lower copy-number noise (0.013 vs 0.11) and higher inferred tumor fraction (0.61 versus 0.48). RNA sequencing succeeded in 14 of 17 cores, with increased base pairs compared to random fresh tissue collection (247 vs 155) and genome alignment rate of 96%. SRH scan number did not affect RNA quality, with no differences in RIN or DV200 among unscanned cores and cores scanned 1-3 times. RNA sequencing failures were associated with small (<7mm biopsy length) and >2 trimmings before biobanking.

Conclusions:
External validation of SRH-AI model enabled rapid PCa confirmation, tumor enrichment, and reliable downstream molecular profiling from MRI targeted biopsy increasing sequencing-suitable cores 5.2-fold. These findings support procedural diagnosis and tissue-acquisition workflows for translational research and precision oncology.

Table 1. Participant Demographics and MRI-Targeting Characteristics

Characteristics	Median (IQR)
Age (years old)	68 (63-74)
PSA (ng/mL)	8.2 (5.7-12.5)
Prostate Vol (mL)	45 (34-64)
PSA density (mg/mL)	0.18 (0.12-0.28)
ROI size (mm)	14 (9-20)
PIRADS (n = 256)	
3	50
4	110
5	96
Initial biopsy core pathology	
Benign	135
GG1	62
GG2	34
GG3	9
GG4	7
GG5	9

PSA: prostate specific antigen. ROI: region of interest. PI-RADS: prostate imaging reporting and data system. GG: grade group.

Table 2. Characteristics of cryobanked biopsy samples for DNA and RNA sequencing

Sample Number	Pathologic grade	Core length (mm)	Number of trimming	SRH-AI predicted cancer area (%)	DNA yield (ng)	RNA yield (ng)	RIN value	DV200	RNA seq	DNA seq
# 1	GG2	8	3	73	125	0	NA	NA		✓
# 2	GG2	15	1	50	5325	660	4.3	93.4	✓	✓
# 3	GG2	11	1	85	1725	885	6.2	95.8	✓	✓
# 4	GG1	13	2	93	4875	848	4.4	91.4	✓	✓
# 5	GG2	15	0	73	5300	1425	6.0	95.2	✓	✓
# 6	GG5	17	0	70	8700	960	5.6	90.7	✓	✓
# 7	GG2	8	3	85	690	0	NA	NA		✓
# 8	GG2	9	2	85	1125	404	3.6	91.0	✓	✓
# 9	GG5	15	0	83	10425	1013	5.2	97.0	✓	✓
# 10	GG2	11	1	85	2475	662	5.5	89.7	✓	✓
# 11	GG3	16	0	86	10800	750	7.5	98.2	✓	✓
# 12	GG2	6	1	86	900	158	2.2	NA		✓
# 13	GG5	15	0	89	4425	343	4.0	97.3	✓	✓
# 14	GG1	12	0	50	3375	530	7.0	98.6	✓	✓
# 15	GG1	11	0	81	2925	750	5.0	96.1	✓	✓
# 16	GG3	17	0	41	4725	578	6.7	96.7	✓	✓
# 17	GG1	13	0	79	5250	1028	6.5	96.8	✓	✓

GG: grade group. SRH: stimulated Raman histology. RIN: RNA integrity number

Acknowledgement of funding: Canadian Institutes of Health Research Project Grant 203748. Canadian Urologic Scholarship Fund. National Institute of Health Grant UL1TR001445.

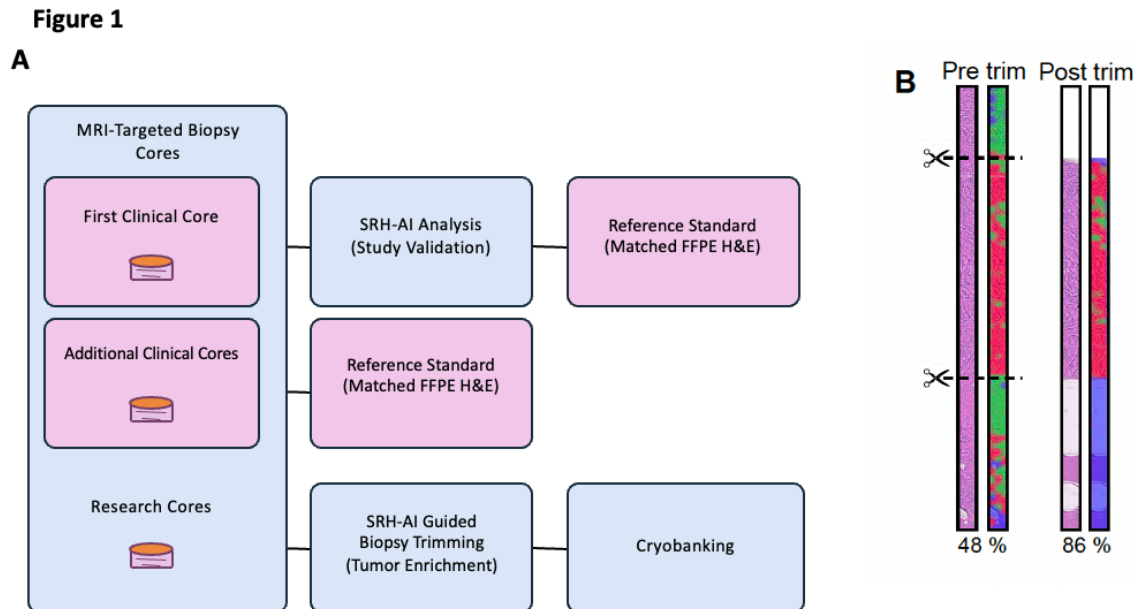


Figure 1. Integration of SRH-AI into the standard prostate biopsy workflow for validation and tumor-enriched cryobanking.

(A) Study workflow. All fresh biopsy cores undergo stimulated Raman histology (SRH) imaging with integrated AI analysis (SRH-AI) to generate a quantitative tumor assessment for study validation. The first clinical core from MRI-targeted biopsy is analyzed using the NYU SRH-AI model, and SRH-AI outputs are compared against the reference standard of matched formalin-fixed paraffin-embedded (FFPE) H&E from the corresponding tissue for model validation. In parallel, additional clinical cores undergo routine processing, with matched FFPE H&E sections serving as the diagnostic reference standard. Research cores are imaged with SRH and analyzed by SRH-AI to localize tumor-enriched regions, which guide biopsy trimming for tumor enrichment; trimmed tumor-enriched tissue is then cryobanked for downstream DNA/RNA sequencing. Blue boxes indicate study-added SRH-AI steps layered onto the clinical workflow, whereas pink boxes indicate the standard-of-care processing and reference pathology workflow. (B) SRH-AI-guided trimming for tumor enrichment. Representative case showing SRH-AI-guided trimming of a research core, with the predicted tumor content increasing from 48% pre-trim to 86% post-trim (dashed lines indicate trim boundaries; scissor icons denote trimming sites). In the SRH image, the base SRH signal is shown in purple, with an AI overlay in which green denotes benign tissue, red denotes cancer, and violet denotes non-diagnostic regions.

Acknowledgement of funding: Canadian Institutes of Health Research Project Grant 203748. Canadian Urologic Scholarship Fund. National Institute of Health Grant UL1TR001445.

Conflicts of Interest: Dr Mannas: Honoraria: Abbvie, Bayer, JnJ, TerSerra. Consultant: Hexamer Therapeutics, JnJ, TerSerra, Tolmar. Research Funding: British Columbia Ministry of Health Innovation Hub, Canadian Institute of Health Research, Canadian Urology Association, Prostate Cancer Foundation, TerSerra, Vancouver Coastal Health Research Institute. The remaining authors have no relevant disclosures.

