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Title: Detection and Isolation of Cancer in Prostate Biopsies Using Stimulated Raman Histology and Artificial Intelligence

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

3. Filming location: Will the filming need to take place in multiple locations? **Yes**. **Another room in the same floor and hallway.**

Current Protocol Length
Number of Steps: 23
Number of Shots: 53

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Miles Mannas:** We aim to integrate artificial intelligence and SRH imaging to detect prostate cancer in near real-time while improving tissue selection for downstream analysis and accelerating diagnosis.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.1*

What are the most recent developments in your field of research?

1.2. **Miles Mannas:** AI models now analyze SRH images in minutes, enabling real-time, label-free cancer diagnosis during diagnostic and therapeutic procedures, reducing reliance on traditional histology and preserving tissue for further analysis.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What technologies are currently used to advance research in your field?

1.3. **Miles Mannas:** SRH, paired with AI and deep learning, enables real-time, label-free tissue imaging and classification, accelerating cancer diagnostics, surgical decision-making, and biomarker discovery without traditional processing or staining.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.11*

What are the current experimental challenges?

1.4. **Miles Mannas:** Ensuring sufficient tumor content in biopsies for accurate genomic profiling, alongside improving SRH-AI alignment with standard pathology, remains a key challenge in achieving diagnostic accuracy in prostate cancer.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.15.2*

What research gap are you addressing with your protocol?

1.5. **Miles Mannas:** We are addressing the need for faster, more accurate prostate cancer diagnosis and better biopsy quality. Our protocol improves tumor detection, increases tumor content, and preserves tissue for molecular testing.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.4*

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please ensure that all testimonial shots are captured in a wide-angle format, while also maintaining sufficient headspace, given that the final videos will be rendered in a 1:1 aspect ratio.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Mingyu Sheng:** Publishing with JoVE will make our protocol more accessible to researchers and clinicians by visually demonstrating each step, helping accelerate adoption of SRH-AI in prostate and urologic cancer diagnostics and biobanking.

1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.7. **Mingyu Sheng:** Publishing with JoVE will help us train collaborators faster and more consistently, improving adoption of our SRH protocol across sites—and potentially speeding up multi-center studies and clinical translation.

1.7.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card
This research has been approved by the Institutional Review Board (IRB) at University of British Columbia

Protocol

2. Guided Imaging Workflow for Prostate Biopsy Using SRH Technology

Demonstrator: Takeshi Namekawa

2.1. To begin, turn on the SRH imager **[1-TXT]**. Attach a 50-milliliter syringe filled with sterilized water to the syringe valve located on the left-hand side of the imager interface **[2]**. Ensure the syringe is securely fitted **[3]**.
- 2.1.1. WIDE: Talent switches on the SRH imager. **TXT: SRH: Stimulated Raman Histology**
- 2.1.2. Talent attaching a syringe filled with sterilized water to the syringe valve on the SRH imager.
- 2.1.3. Shot of the syringe being checked for fitting.

2.2. Once the system loads, use the touchscreen monitor to enter the **Username** and **Password** then tap on **Log In [1]**. Accept the **Disclaimer** to acknowledge that the study is for research purposes if used outside of the United States or European Union **[2]**.
- 2.2.1. SCREEN: 68083_screenshot.mp4.mp4 00:00-00:12
- 2.2.2. SCREEN: 68083_screenshot.mp4.mp4 00:12-00:22

2.3. Now, select **Create New Study** from the display options **[1]**. After entering the case information, save the samples under an appropriate file name such as John Doe **[2]**.
- 2.3.1. SCREEN: 68083_screenshot.mp4.mp4 00:22-00:32
- 2.3.2. SCREEN: 68083_screenshot.mp4.mp4 00:32-00:42
  Video Editor: Please highlight the "Last name, First name" field

2.4. Under **Primary Anatomical Location**, select **Prostate (Research Use Only)** *(Prostate-Research-Use-Only)* **[1]**. For **Analysis Module**, choose **Prostate Cancer (Research Use Only)** to enable the integrated artificial intelligence for prostate cancer detection **[2]**.
- 2.4.1. SCREEN: 68083_screenshot.mp4.mp4 00:42-00:52
  Video Editor: Please highlight the Primary Anatomical Location row
- 2.4.2. SCREEN: 68083_screenshot.mp4.mp4 00:52-01:02
  Video Editor: Please highlight the Analysis Module Location row

2.5. Now tap **Create Study** after entering the case information **[1]**. When prompted, tap **Acknowledge** to confirm that the data will be used for research purposes only **[2]**.
- 2.5.1. SCREEN: 68083_screenshot.mp4.mp4 01:02-01:12
- 2.5.2. SCREEN: 68083_screenshot.mp4.mp4 01:12-01:22
  Video Editor: Please highlight the "Acknowledge" option

2.6. Fill the fluid chamber with sterilized water as instructed **[1]**. Tap **Next** when prompted, then select **Acquire** from the options displayed **[2]**. Now press **Load New Specimen** to prompt the system to display **Prepare Specimen & Load NIO** *(N-I-O)* **Slide [3]**.
- 2.6.1. Talent filling the fluid chamber with sterilized water.
- 2.6.2. SCREEN: 68083_screenshot.mp4.mp4 01:22-01:32
  Video Editor: Please highlight the "Next" option
- 2.6.3. SCREEN: 68083_screenshot.mp4.mp4 01:32-01:42
  Video Editor: Please highlight the "Load New Specimen" option

2.7. Retrieve the prostate biopsy slide **[1]**. Using tissue forceps with teeth, open the attached coverslip **[2]** and place the prostate biopsy from the RPMI media securely in the groove of the slide **[3]**. Gently close the coverslip and secure the sample **[4]**.
- 2.7.1. Talent retrieving biopsy slide.
- 2.7.2. Shot of the coverslip being removed with tissue forceps.
- 2.7.3. Talent using forceps to transfer biopsy to slide groove.
- 2.7.4. Talent closing coverslip to secure sample.

2.8. Open the slide holder on the SRH imager interface **[1]** and insert the prepared slide, ensuring proper alignment **[2]**. Close the lid and tap **Next** when the system detects closure **[3]**.
- 2.8.1. Talent opening slide holder on the SRH imager.
- 2.8.2. Talent inserting the prepared slide.
- 2.8.3. Talent closing the lid and tapping Next on screen.
  VIDEOGRAPHER'S NOTE: Place shot 2.8.3 after 2.9.2

2.9. Set the imaging parameters by choosing **Biopsy 1 A** for **Specimen Name**, **0.4 mm x 6.1 mm** *(0 point 4-millimeters-into-6-point-one-millimeters)* for **Scan Area** and choose **3 regions (Research Use Only) [1]**. For the **Scan Position**, manually adjust the scan location using the on-screen image **[2]**. Then tap **Acquire Image** to proceed **[3]**.
- 2.9.1. SCREEN: 68083_screenshot.mp4.mp4 01:42-01:52
- 2.9.2. Talent adjusts the scan location using on-screen image.
- 2.9.3. SCREEN: 68083_screenshot.mp4.mp4 01:52-02:02
  Video Editor: Please highlight the "Acquire Image" option

2.10. Review the sample details on screen and confirm by selecting **Proceed [1]**.
- 2.10.1. SCREEN: 68083_screenshot.mp4.mp4 02:02-02:12

2.11. Let the imager combine sections to generate a SRH image **[1]**. Then apply the artificial intelligence overlay by clicking the **>>** *(greater than)* icon to highlight cancer regions in red, non-cancer regions in green, and non-diagnostic regions in violet **[2]**.
- 2.11.1. SCREEN: 68083_screenshot.mp4.mp4 02:12-02:36
- 2.11.2. SCREEN: 68083_screenshot.mp4.mp4 02:36-02:46
  Video Editor: Please highlight the ">>" option

2.12. Now review the bar graph showing the percentage of cancerous, non-cancerous, and non-diagnostic tissues **[1]**. Click the **<<** *(lesser than)* icon to return to the original SRH image without the artificial intelligence overlay **[2]**.
2.12.1. SCREEN: 68083_screenshot.mp4.mp4 02:46-02:56
Video Editor: Please highlight the bar graph
2.12.2. SCREEN: 68083_screenshot.mp4.mp4 02:56-03:06
Video Editor: Please highlight the "<<" option

2.13. Use the **Zoom-in** and **Zoom-out** functions to examine the biopsy image in detail **[1]**. Then, use the navigation icons to examine the biopsy image **[2]**.
2.13.1. SCREEN: 68083_screenshot.mp4.mp4 03:06-03:15,03:22-03:25
2.13.2. SCREEN: 68083_screenshot.mp4.mp4 03:35-03:42

2.14. After initial scanning, use tissue forceps with teeth to lift the coverslip **[1]** and gently remove the biopsy from the slide **[2]**.
2.14.1. Shot of the coverslip being lifted.
2.14.2. Talent removes the biopsy from the slide.

2.15. Transfer the biopsy onto a moistened Telfa soaked in saline to maintain tissue integrity **[1]**. With a surgical blade, trim the non-cancer regions based on the artificial intelligence overlay to improve the cancer-to-tissue ratio, typically focusing on the ends **[2]**.
2.15.1. Talent placing biopsy on saline-moistened Telfa.
2.15.2. Talent trimming biopsy with surgical blade.

2.16. Re-scan the trimmed biopsy **[1]**. Adjust the scan area and position to confirm the increased cancer-to-tissue ratio **[2]**.
2.16.1. Talent preparing to re-scan biopsy
2.16.2. SCREEN: 68083_screenshot.mp4.mp4 03:42-03:58

2.17. Now remove the biopsy from the slide using tissue forceps **[1]** and place it into a cryotube **[2]**. Then freeze the cryotube in liquid nitrogen **[3]**.
2.17.1. Shot of biopsy being removed from the slide.
2.17.2. Shot of the biopsy being transferred into a cryotube.
2.17.3. Talent place the cryotube in liquid nitrogen.

~~2.18. Transfer the cryotube to a minus 80-degree Celsius freezer or liquid nitrogen storage for long-term preservation **[1]**.~~
~~2.18.1. Talent placing cryotube at – 80 °C.~~
AUTHOR'S NOTE: Shot not filmed since it is routine

2.19. To export the image data, press the **paper airplane** icon to begin exporting image data **[1]**. Tap **Select Export Location** and choose **USB (Complete)** *(U-S-B-Complete)*, then select the external hard disk **[2]**.

2.19.1. SCREEN: 68083_screenshot.mp4.mp4 03:58-04:07

Video Editor: Please highlight the "Paper plane" option (lower right)

2.19.2. SCREEN: 68083_screenshot.mp4.mp4 04:07-04:17

2.20. Now select **Entire Study** to export all SRH image series **[1]**. Wait for the **Export in Progress** message and tap **Confirm** when complete **[2]**.

2.20.1. SCREEN: 68083_screenshot.mp4.mp4 04:17-04:27

Video Editor: Please highlight the "Entire Study" option

2.20.2. SCREEN: 68083_screenshot.mp4.mp4 04:27-04:37

Video Editor: Please highlight the "Confirm" option when VO says Confirm

2.21. To turn off the instrument, press **Exit** and follow the shutdown instructions on screen **[1]**. Select **Proceed Without** when prompted to archive data if already exported **[2]**.

2.21.1. SCREEN: 68083_screenshot.mp4.mp4 04:37-04:47

Video Editor: Please highlight the "Exit" option

2.21.2. SCREEN: 68083_screenshot.mp4.mp4 04:47-04:57

Video Editor: Please highlight the "Proceed Without" option

2.22. Dispose of the sample following lab protocol **[1]**. Then tap **Next [2]** and use the attached syringe to empty the fluid chamber **[3]**.

2.22.1. Talent removes and disposes the sample.

2.22.2. SCREEN: 68083_screenshot.mp4.mp4 04:57-05:07

Video Editor: Please highlight the "Next" option

2.22.3. Talent uses attached syringe to empty the fluid chamber.

2.23. Tap **Next** again, then select **Yes** from the display options **[1]**. Now remove and dispose of the syringe **[2]**. Then press **Shut Down** to turn off the SRH microscope **[3]**.

2.23.1. SCREEN: 68083_screenshot.mp4.mp4 05:07-05:17

2.23.2. Talent removes and disposes of syringe.

2.23.3. SCREEN: 68083_screenshot.mp4.mp4 05:17-05:27

Results

3. Representative Results

3.1. Three distinct scans were performed across the biopsy specimen to generate a pseudo-hematoxylin and eosin-stained stimulated Raman histology image **[1]**.

3.1.1. LAB MEDIA: Figure 2B

3.2. Artificial intelligence overlay on the SRH image differentiated tumor, non-tumor, and non-diagnostic regions using red, green, and violet segments respectively **[1]**.

3.2.1. LAB MEDIA: Figure 3A and B *Video editor: Please sequentially highlight tumor, non-tumor and non-diagnostic regions of A and B*

3.3. After trimming non-diagnostic areas, rescanned biopsies revealed an increased tumor proportion from 27% to 72% **[1]**.

3.3.1. LAB MEDIA: Figure 3D-E *Video editor: Please highlight non-tumor and Tumor regions of both D and E*

3.4. Cancer-to-tissue ratio significantly increased after trimming, with average cancer percentage rising from 45% precut to 78% postcut across 46 biopsies **[1][2]**.

3.4.1. LAB MEDIA: Figure 4B. *Video editor: Please highlight the blue box then the red box*

3.5. Suboptimal scanning at 10 micrometer depth without ink resulted in lower image clarity due to improper parameter settings **[1]**. Imaging of inked margins introduced visual artifacts, presenting as darkened or unclear regions due to interference with laser signal acquisition **[2]**.

3.5.1. LAB MEDIA: Figure 5A.

3.5.2. LAB MEDIA: Figure 5B.

Pronunciation Guide:

1. Histology

Pronunciation link:
https://www.merriam-webster.com/dictionary/histology
IPA: /hɪˈstɑːlədʒi/
Phonetic Spelling: hiss-taa-luh-jee

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2. Biopsy

Pronunciation link:
https://www.merriam-webster.com/dictionary/biopsy
IPA: /ˈbaɪˌɑːpsi/
Phonetic Spelling: bai-aap-see

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3. Cryotube

Pronunciation link:
https://www.howtopronounce.com/cryotube
IPA: /ˈkraɪ.oʊ.tjuːb/
Phonetic Spelling: krai-oh-toob

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4. Telfa

Pronunciation link:
https://www.howtopronounce.com/telfa
IPA: /ˈtɛl.fə/
Phonetic Spelling: tel-fuh

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5. Stimulated Raman Histology

Pronunciation link:
https://www.howtopronounce.com/stimulated-raman-histology
IPA: /ˈstɪmjəˌleɪtɪd ˈrɑːmən hɪˈstɑːlədʒi/
Phonetic Spelling: stim-yuh-lay-tid rah-mahn hiss-taa-luh-jee

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6. Syringe

Pronunciation link:
https://www.merriam-webster.com/dictionary/syringe
IPA: /səˈrɪndʒ/
Phonetic Spelling: suh-rinj

7. Specimen

Pronunciation link:
https://www.merriam-webster.com/dictionary/specimen
IPA: /ˈspɛsəmɪn/
Phonetic Spelling: speh-suh-min

8. RPMI

Pronunciation link:
https://www.howtopronounce.com/rpmi
IPA: /ɑːr.piː.em.aɪ/
Phonetic Spelling: ar-pee-em-eye

9. NIO

Pronunciation link:
https://www.howtopronounce.com/nio
IPA: /ˈnaɪ.oʊ/
Phonetic Spelling: nai-oh

10. Overlay

Pronunciation link:
https://www.merriam-webster.com/dictionary/overlay
IPA: /ˈoʊ.vɚ.leɪ/
Phonetic Spelling: oh-ver-lay

11. Artificial

Pronunciation link:
https://www.merriam-webster.com/dictionary/artificial
IPA: /ˌɑːrtəˈfɪʃəl/
Phonetic Spelling: ar-tuh-fish-uhl

12. Imager

Pronunciation link:
https://www.howtopronounce.com/imager
IPA: /ˈɪm.ɪ.dʒɚ/
Phonetic Spelling: ih-muh-jer