FINAL SCRIPT: APPROVED FOR FILMING

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Title: Microarray Integrated Spatial Transcriptomics (MIST) as a Scalable, Cost-Effective Solution for High-Throughput Spatial Profiling

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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, 4 km apart**

Current Protocol Length
Number of Steps: 12
Number of Shots: 29

Introduction

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

1.1. **Juwayria:** Our research focuses on overcoming restricted sample usage and high cost in spatial transcriptomics by integrating tissue microarrays with spatial platforms like 10X Visium using low-cost custom laser-cut designs assisted by inexpensive 3D printing.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.11.1*

Videographer's Note: C0020-23 : Test shots, C0024 : Final Take

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

1.2. **Ishaan Gupta:** Digital Pathology aided by Spatial transcriptomics assays has seen an explosion in the last two years with many research and commercial solutions coming up. But the cost of these assays is still very high which limits their adoption in low-resource settings.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.3*

Videographer's Note: C0032 : Final Take

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

1.3. **Ishaan Gupta:** There are 2 major spatial transcriptomics technologies used - first based on capturing and barcoding the molecules on a slide followed by sequencing with the capacity to detect all potential transcripts; second based on proximity ligation and in-situ imaging of a limited set of transcripts.

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

Videographer's Note: C0034: Final Take

What research gap are you addressing with your protocol?

1.4. **Juwayria:** We address the lack of affordable, scalable, and customizable solutions for high-throughput spatial transcriptomics, especially for biopsy-limited samples such as core needle biopsies in low-resource settings.

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

Videographer's Note: C0027 : Final Take

What research questions will your laboratory focus on in the future?

1.5. **Prabhat Singh Malik:** With cheaper access to the technology we aim to pursue research into understanding intra-tumor and intra-individual heterogeneity in cancer patients towards a better understanding of disease progression and prognosis.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera
Videographer's Note: C0050 : Final Take

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

Ethics Title Card

This research has been approved by the Institute Ethics Committee at the All India Institute of Medical Sciences, New Delhi

2. Fabrication of Custom Tissue Microarray Blocks Using Laser-Cut Stencils and 3D-Printed FFPE Holders

Demonstrator: Juwayria, Kaustar Yadav

2.1. To begin, select a 1-to-2-centimetre thick polymethyl methacrylate sheet for laser cutting **[1-TXT]**.

2.1.1. WIDE: Talent selecting a 1 - 2 cm thick acrylic PMMA sheet from a stack. **TXT: Do not use temperature sensitive polycarbonate sheets**
Videographer's Note: C0003 : Final Take

2.1.2. Talent examining thicker 1.5 to 2 centimeter acrylic sheets on the bench.
Videographer's Note: C0002 : Final Take

2.2. Launch the RDWorks *(R-D-Works)* software and upload the provided .DXF *(Dot-D-X-F)* file to begin creating the microarray design **[1]**. If the .DXF file is not available, manually input circular cores into RDWorks and adjust the coordinates from the top panel **[2]**.

2.2.1. SCREEN: 68516_2.2.1_2.3.1_2.3.2.mp4. 00:00-00:16

2.2.2. SCREEN: 68516_2.2.2.mp4. 00:04-00:16,00:25-00:38

2.3. Set the laser cutter to operate at 50% laser power, corresponding to 100-watt power of the 200-watt carbon dioxide laser's capacity **[1]**. Adjust the cutting speed to 40 millimeters per second for precise and efficient material removal **[2]**. Use a laser with a spot diameter of 0.1 millimeter to minimize edge distortion and achieve high precision **[3]**.

2.3.1. SCREEN: 68516_2.2.1_2.3.1_2.3.2.mp4. 00:28-00:44

2.3.2. SCREEN: 68516_2.2.1_2.3.1_2.3.2.mp4. 00:22-00:27

2.3.3. Shot of the laser lens specification showing the 0.1-millimeter spot diameter.
Videographer's Note: C0004: Wide shot of the laser machine, C0005-06 : Final Take

2.4. Secure the acrylic sheet onto the laser cutting platform **[1].** Start the laser cutting process using the configured parameters to fabricate the stencil **[2]**. Then reset the origin coordinates to original position for accurate alignment **[3]**.
NOTE: VO edited to reflect order of moved shots.

2.4.1. Talent placing the PMMA sheet on the laser cutter.
Videographer's Note: C0007 : Final Take

2.4.2. Talent doing 'reset' of the laser coordinates using the control panel.
Videographer's Note: C0008 : Final Take, C0009: Laser being reset
AUTHOR'S NOTE: Move 2.4.2 after 2.4.3

2.4.3. Talent initiating laser cutting and monitoring the stencil fabrication.
Videographer's Note: C0010: Talent initiating laser cutting (part-a), C0011: Monitoring the stencil fabrication (part b), C0012: Better angle for part b, C0040: Result after laser Cut , The lid of machine was open only for filming purposes

2.5. Next, design the FFPE *(F-F-P-E)* block holder in SolidWorks *(Solid-Works)* and export the computer-aided design file as an STL *(S-T-L)* file using the software's built-in export feature **[1-TXT]**.

2.5.1. SCREEN: 68516_2.5.1.mp4. 00:03-00:29
TXT: Use provided design file for direct use

2.6. Use a fused deposition modelling 3D printer such as Creality for the printing process **[1]**. Load the STL file into the slicing software and generate the G-code required for printing **[2]**. Ensure the infill setting is at 100 percent to produce a solid and durable structure **[3]**.

2.6.1. Talent powering on the FDM 3D printer.
Videographer's Note: C0013 : Final Take

2.6.2. SCREEN: 68516_2.6.2_2.6.3.mp4. 00:21-00:38, 01:44-01:52

2.6.3. SCREEN: 68516_2.6.2_2.6.3.mp4. 01:10-01:20

2.7. Select acrylonitrile butadiene styrene filament as the material for 3D printing the FFPE block holder **[1]**. Then start the 3D printing process using the configured parameters and monitor the print for proper adhesion and layer deposition **[2]**. Once printing is complete, allow the FFPE block holder to cool before carefully removing it from the print bed **[3]**.

2.7.1. Talent loading ABS filament into the printer and checking the spool label.
Videographer's Note: C0016 : Final Take

2.7.2. Shot of 3D printing in progress.
Videographer's Note: C0017-18 : Shot of 3d printing in progress

2.7.3. Talent removing the cooled FFPE block holder gently from the print bed.
Videographer's Note: C0036-36 : Multiple shots to choose from

2.8. To make the tissue microarray block, first prepare a blank paraffin block with dimensions larger than the spatial slide's capture area **[1-TXT]**. Place the customized stencil on top of the paraffin block and align it accurately for core placement **[2]**. The, use a 2-millimeter drill bit to create cylindrical cavities through the paraffin either manually or with an automated pen drill **[3]**.

2.8.1. Talent measuring and positioning a blank paraffin block on the workbench. **TXT: For capture area of 6.5 x 6.5 mm, prepare block of 1.5 - 2 cm**
Videographer's Note: C0044-46 : Multiple Takes

2.8.2. Talent aligning the laser-cut stencil onto the paraffin block.
Videographer's Note: 2.8.2-2.8.3 : Filmed together. C0050-51 : Final Take

2.8.3. Talent drilling cylindrical holes through the stencil into the block using an automated 2-millimeter pen drill.

2.9. Next, use a 2-millimeter coring tool to extract cylindrical tissue cores from the marked regions of interest on the FFPE donor block **[1]**. ~~Insert the extracted tissue cores into the corresponding cylindrical cavities in the microarray block, making sure they fit snugly **[2]**.~~
NOTE: Shot 2.9.2 deleted by authors

2.9.1. Talent identifying and extracting tissue cores from the donor block using a 2-millimeter coring tool.

~~2.9.2. Talent positioning the core over the microarray block and inserting it into a cavity.~~

2.10. Hold the punch perpendicular to the block surface, apply downward pressure to pierce the paraffin **[1]**, then gently retract the tool with the core embedded **[2]**. After retraction, manually release the tissue core into the cavity **[3]**.

2.10.1. Shot of the punch being held vertically then pressed down.

2.10.2. Talent retracting the punch with the embedded tissue.

2.10.3. Shot of the core being released into the cavity.

2.11. Incubate the completed microarray block on a digital hot air oven set to 45 degrees Celsius for 1 hour to enhance adhesion between the paraffin and tissue cores **[1]**. After incubation, cool the block at 4 degrees Celsius for 15 to 20 minutes to solidify the paraffin **[2]**.

2.11.1. Talent placing the microarray block onto a digital hot air oven for 1 hour.

2.11.2. Talent transferring the incubated block to a refrigerated unit at 4 degrees Celsius.

2.12. Trim the surface of the block using a microtome for a smooth and even finish **13]**. Then, place the trimmed block ~~at 4 degrees Celsius for 5 minutes **[2]** to stabilize it before moving to~~ in long-term storage at 4 degrees Celsius **[3]**.
NOTE: Shot 2.12.2 deleted by authors

2.12.1. Talent trimming the surface of the block with a microtome.

~~2.12.2. Shot of the block being placed at 4 °C.~~

2.12.3. Talent placing the trimmed block in cold storage labeled for long-term use at

4 degrees Celsius.

Results

3. Results

3.1. Calcified tissue cores are hard textured and difficult to section, compromising structural integrity and leading to unusable sections **[1]**. When initial core insertion was uneven, reinsertion of a new core was required to maintain sampling consistency across the tissue microarray **[2]**.

3.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the top left circle*

3.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight the top right circle*

3.2. Tissue cores that were not uniformly cylindrical or had air bubbles during paraffin processing led to incomplete sections or failed extractions [**1].**

3.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight the bottom right circle*

3.3. Visium Spatial Transcriptomics enabled analysis at near single-cell resolution, with each 50 micrometre spot capturing between 1 and 10 cells **[1]**.

3.3.1. LAB MEDIA: Figure 3. *Video editor: Highlight the image labelled "Traditional ST"*

3.4. Despite the reduced number of spots in MIST *(mist)*, SpaCET *(Space-Set)* deconvolution achieved accurate cell type annotations **[1]**.

3.4.1. LAB MEDIA: Figure 3. *Video editor: Highlight the image labelled " inhouse developed Microarray integrated ST" and "Automated gene-based annotation of ST data".*

3.5. Manual pathologist annotation marked tumor regions in green on hematoxylin and eosin -stained sections, establishing a reference for comparison **[1]**. Automated gene-based spatial transcriptomics annotations of tumor cells showed high concordance with manual annotations, validating the analytical approach **[2]**.

3.5.1. LAB MEDIA: Figure 3. *Video editor: Show the image labelled "Manual annotation of tumor regions by Pathologist"*

3.5.2. LAB MEDIA: Figure 3. *Video editor: Highlight the image labelled"Automated gene-based annotation of ST data".*

Pronunciation Guide:

1. Microarray

- **Pronunciation link: https://www.howtosay.co.in/pronounce/microarray-in-english/**
- **IPA: /ˌmaɪkroʊˈæreɪ/**
- **Phonetic Spelling: my-kroh-AIR-ay**

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2. Transcriptomics

- **Pronunciation link: While no exact site was found, you can treat it as "transcript + -omics." ("omics" rhymes with "phoenix" minus 'ph', pronounced /ˈoʊmɪks/)**
- **IPA: /træns.krɪpˈtoʊmɪks/**
- **Phonetic Spelling: trans-krip-TOH-miks**
  (This follows standard American pronunciation patterns.)

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3. Spatial

- **Pronunciation link: https://www.merriam-webster.com/dictionary/spatial (implied)**
- **IPA: /ˈspeɪʃəl/**
- **Phonetic Spelling: SPAY-shəl**

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4. Transcriptomics *(repeat for consistency)*

— As above —

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5. FFPE (Formalin-Fixed Paraffin-Embedded)

- **Pronunciation link: Not an English word—it's an acronym.**
- **IPA: /ɛf-ɛf-pi-iː/**
- **Phonetic Spelling: eff-eff-PEE-ee**

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6. Fabrication

- **Pronunciation link: https://www.merriam-webster.com/dictionary/fabrication (implied)**
- **IPA: /ˌfæbrɪˈkeɪʃən/**
- **Phonetic Spelling: fab-ri-KAY-shun**

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7. Acrylonitrile butadiene styrene (ABS)

- **Pronunciation link: https://www.merriam-webster.com/dictionary/acrylonitrile (implied)**
- **IPA for "acrylonitrile": /ˌækrələʊˈnɪtraɪl/**
- **Phonetic Spelling: ak-ri-loh-NIGH-trile**

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8. Paraffin

- **Pronunciation link: https://www.merriam-webster.com/dictionary/paraffin (implied)**
- **IPA: /ˈpærəfɪn/**
- **Phonetic Spelling: PAIR-uh-fin**

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9. Biopsy

- **Pronunciation link: https://www.merriam-webster.com/dictionary/biopsy (implied)**
- **IPA: /ˈbaɪɑːpsi/**
- **Phonetic Spelling: BY-op-see**

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10. Hematoxylin

- **Pronunciation link: https://www.merriam-webster.com/dictionary/hematoxylin (implied)**
- **IPA: /ˌhiːmətəˈzɪlɪn/**
- **Phonetic Spelling: hee-muh-TOX-i-lin**