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FFPE Human Brain Tissue Serial Sectioning for Xenium

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Wenqing Cao¹, Tsering Lama¹, Xiaoying(Miya) Lai¹, Philip De Jager¹, Mariko Taga¹, YA ZHANG¹

¹The Center for Translational & Computational Neuroimmunology, Department of Neurology, Columbia University

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We use this protocol and it's working

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Abstract

The Xenium In Situ Gene Expression platform from 10x Genomics enables high-plex, targeted RNA detection at subcellular resolution, supporting spatially resolved cell typing and molecular profiling. While an optimized tissue preparation protocol was provided by 10x, formalin-fixed paraffin-embedded (**FFPE**) **post-mortem human brain tissue** presents unique challenges due to its high lipid content. Organic solvents used during paraffin embedding extract lipids from myelin-rich white matter, leading to membrane disruption and sectioning artifacts such as compression, chatter, and fragmentation. In addition, the Xenium platform provides slides with a special coating and a defined 10.45 mm × 22.45 mm capture area, which demand precise handling during tissue placement to maximize the utilization of the capture area and ensure tissue quality.

In this protocol, we demonstrate the procedure for attaching **three 6 mm × 8 mm** sections of human brain tissue. This protocol outlines a standardized workflow with quality control checkpoints and troubleshooting to preserve morphology, minimize artifacts, and ensure reproducibility for high quality Xenium assays on FFPE human brain tissue.

Guidelines

This protocol consists of steps including preparation of Xenium slides, microtome workspace and flotation bath, block facing, rehydration, serial sectioning, region of interest (ROI) cutting, and tissue attachment onto Xenium capture slides.

Materials

1. HistoCore AUTOCUT – Leica
CN: 149AUTO00C1, 14051956472
2. Tissue Floating Bath, Lighted – Geyer Instructional Products
SKU: 194242
3. Epredia™ Ultra Disposable Microtome Blades – Fisher Scientific
CN: 3053835
4. Camel Hair Brushes, #1, less than 1.59mm wide – Ted Pella
CN: NC1588898
5. Fisherbrand General-Purpose Pinning Forceps – Fisher Scientific
CN: 10-270
6. Fisherbrand High Precision Straight Slender Fine Point Tweezers/Forceps – Fisher Scientific
CN: 12-000-127
7. Surgical Design Surgeon Grade Scalpel Handles #3 – Fisher Scientific
CN: 13-812-234
8. Surgical Design No. 10 Carbon Scalpel Blade – Fisher Scientific
CN: 22-079-690
9. Well-Tech self-healing PVC cutting mat, 15 × 20 cm – Rave Scientific
RS-MN-15-008054
10. Nalgene™ Acrylic Desiccator Cabinets – Thermo Fisher Scientific
CN: 5317-0120

Safety warnings

 Be careful when positioning and cleaning the microtome blades.

Ethics statement

All brain specimens were derived from two longitudinal clinico-pathological cohort studies, that is, the ROS and MAP. In both cohorts, participants did not have known dementia at the time of enrollment. Participants agreed to receive clinical evaluation each year and to donate their brain at the time of death. Each study was approved by the institutional review board of Rush University Medical Center. All participants signed a written informed consent, Anatomical Gift Act and repository consent.

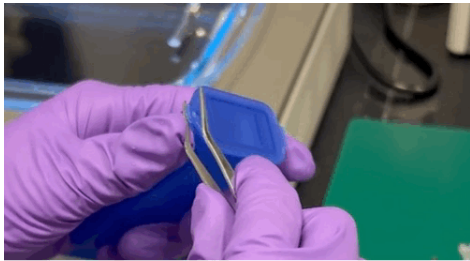
Before start

Quality checks must be performed prior to sectioning for Xenium slides. Refer to the “**FFPE Human Brain Tissue Quality Control for Xenium**” protocol for detailed procedures.

Preparation

1 Xenium Slides Preparation:

- 1.1 Remove the Xenium slide from the -20 °C freezer and allow it to **equilibrate to room temperature** for at least **30 min.**
- 1.2 Open the slides mailer by inserting forceps into the gap of the lid to pry it.



Open the slides mailer with forceps

Note

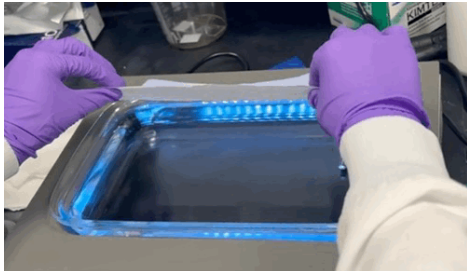
Once the mylar packaging is opened, the unused slides can be stored in the desiccator for up to 7 days. **DO NOT put it back in the freezer.**

2 Microtome Preparation:

- 2.1 Clean the workspace and microtome thoroughly using 70% ethanol followed by RNaseZap. Use 100% ethanol to remove any residual paraffin.
- 2.2 Set the microtome blade angle to **10 degrees**. Adjust the sectioning thickness to **20 µm for facing and trimming**, and **5 µm for serial sectioning**.

3 Flotation Bath Preparation:

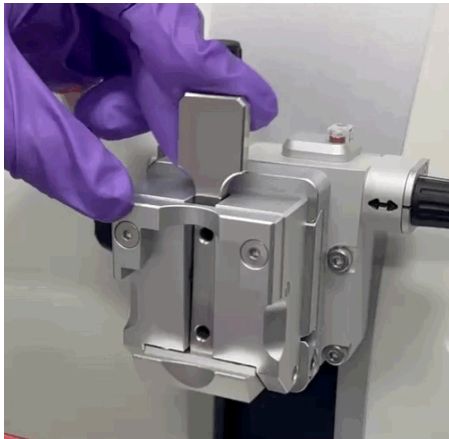
- 3.1 Fill the flotation bath with **Milli-Q water** to just below the rim. Turn on the bath light and set the water temperature to **42 °C**.
- 3.2 Drape Kimwipes gently over the water surface to remove air bubbles and lint. Repeat the process as needed.



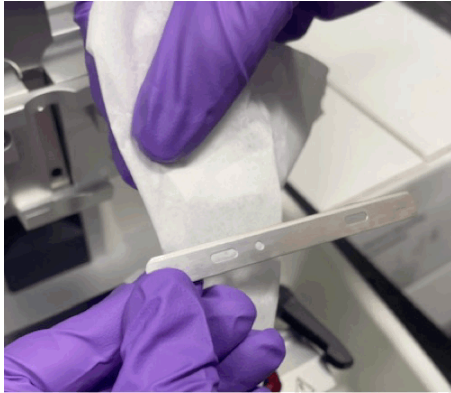
Remove the lint and bubbles from flotation bath

Facing the Block

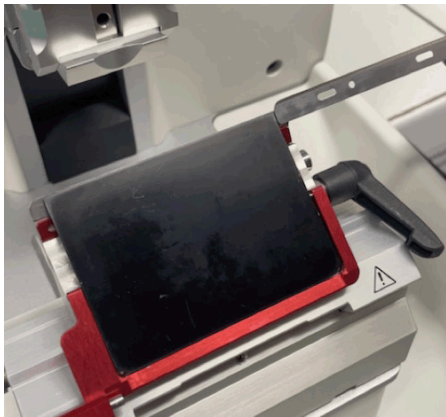
- 4 Scrape away any excess paraffin from the sides of the FFPE block. Secure the block firmly into the microtome specimen holder.



- 5 Wipe off any residual oil from the microtome blade.



- 6 Insert the blade into the blade holder and ensure it is correctly aligned and seated without any tilt.



Note

Low-profile blade must be positioned with the red insert.

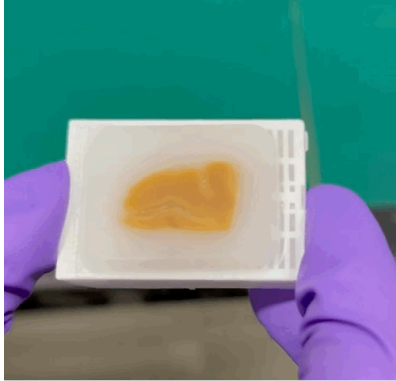
- 7 Adjust the specimen holder until it reaches the original starting position and switch to the **TRIM** mode.
- 8 Move the blade holder base toward the block until the blade just contacts the block surface.

- 9 Slowly rotate the handwheel clockwise to trim the block surface until the entire tissue area or region of interest (ROI) is fully exposed.



Note

Unexposed tissue remains covered with paraffin, which appears lighter, more opaque, and slightly glossy. Fully exposed tissue shows a clearer texture and a darker, more defined color tone.



Example of fully exposed tissue block

Rehydration

- 10 Add 100ml nuclease-free water into a 250ml beaker. Place the beaker on ice and fully submerge the tissue block in the cold water for rehydration.



- 11 Rehydration time may vary from **40 to 90 min**, depending on the quality of the tissue block.
- 12 Collect one section and transfer it to the flotation bath. A section that floats smoothly without wrinkles or air bubbles indicates successful rehydration.

Sectioning

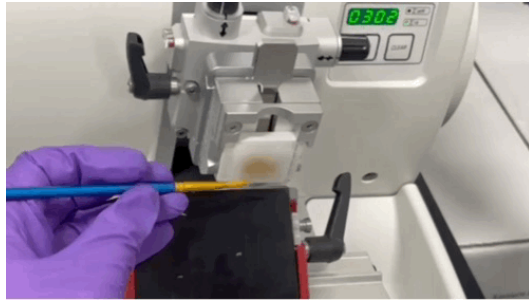
- 13 Place the block back to the specimen holder and adjust the position. Place a new blade and switch to the **SECT** mode.

Note

Always position the tissue block in the same orientation when placing it into the specimen holder. Additionally, take advantage of the coarse feed wheel (black handle on the left) to adjust the position of the specimen holder. The coarse feed wheel allows more control and efficiency when repositioning.

- 14 Slowly rotate the handwheel clockwise and guide the ribbon up and away from the blade using a paintbrush or forceps until the ribbon reaches the targeted length. Discard the

first few sections before forming a tissue ribbon.

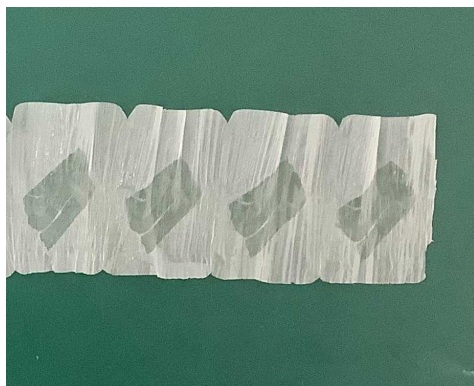


Guide the sections with the paintbrush

Note

The key to forming long tissue ribbons is maintaining the proper speed and directing the ribbon toward your chin as you section.

- 15 Transfer the ribbon to the cutting mat faced up for better visualization of the tissue.



Faced up ribbon with a clear tissue boundary

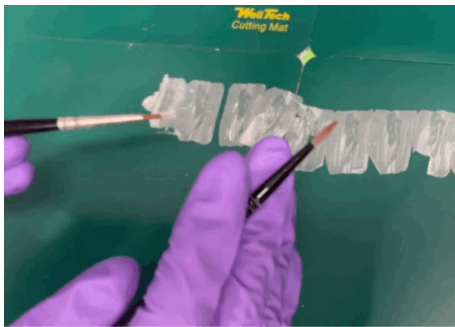
ROI Cutting and Tissue Attachment

- 16 Use a scalpel to cut the ROI from the ribbon



Cut the ROI based on previous selection

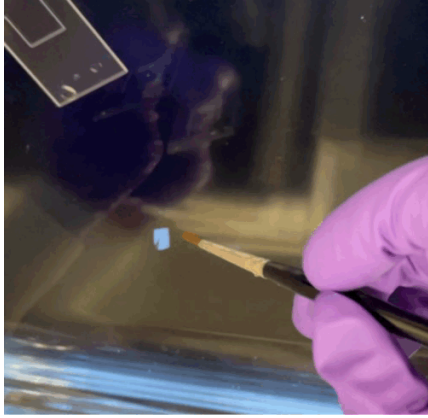
- 17 Use paintbrushes to transfer each ROI section into the water. Allow the tissue to fully expand.



Note

Cut the ROI and transfer to the Xenium slides within 10 min to avoid tissue dehydration.

- 18 Dip the Xenium slide into the flotation bath. Using a **cold paintbrush** to maneuver the ROI section onto the top of the capture area and slowly lift the slide for attachment.



- 19 Transfer the second and third ROI tissue into the water and repeat the mounting step. Make sure the previously mounted sections do not submerge again.
- 20 Leave the Xenium slide at **room temperature** until the sections are completely **dry**.
- 21 Transfer the slide to a **42°C** incubator for **3 hr**, followed by **overnight** drying in a desiccator with relative humidity maintained below **10%**.
- 22 Refer to the following protocols from 10x Genomics for the subsequent steps
 - Xenium In Situ for FFPE – Deparaffinization & Decrosslinking;
 - Xenium In Situ Gene Expression User Guide;
 - Xenium Analyzer User Guide.

Troubleshooting

23 Poor tissue adhesion during section mounting

Cause: The Xenium slides are cold

Troubleshooting: Ensure the Xenium slides are equilibrated to room temperature for enough time prior to use, or briefly immerse the slides in the water bath before mounting. Selecting the ROI containing paraffin can also enhance the adhesion of the tissue to the slide

24 Accidentally cover a fiducial on Xenium slide

Cause: Tissue sections are larger than the fiducial frame or impossible to submerge the slide into the water bath again to reposition the sections

Troubleshooting: Continue the sample preparation workflow up to the point prior to loading the slide cassette onto the instrument. Remove liquid from the cassette and take the slides out. Gently scrape off any tissue covering the fiducials using a pipette tip. Return the slides to the cassette, add 1000 μ L of PBS-T, and then proceed with the Xenium run.

25 **Small cuts on tissue sections when forming ribbon**

Cause: When sectioning to form tissue ribbons, small cuts may appear on the tissue due to paraffin build up or nicks on blade, how the tissue ribbon is held when sectioning, and insufficient rehydration.

Troubleshooting:

As you section new tissue blocks, routinely clean the front and back of the blade with 100% Ethanol to dissolve any paraffin build up or move the blade holder to the left or right to expose a new part of the blade. Additionally, when forming long ribbons, ensure the ribbon is directed up and away from the blade (towards your chin) for uniform sections. If these adjustments during sectioning fail to solve the problem, this may point to insufficient rehydration of the tissue block. We recommend rehydrating the block back into the beaker of water for another 15 min or until the surface of the tissue is light in color and the paraffin is glowing.

26 **Tissue ribbon is not forming**

Cause: Handling of the tissue ribbon, a dull blade, and insufficient rehydration of the block may prevent the formation of a ribbon as you section.

Troubleshooting:

When forming tissue ribbons, how the paintbrush is positioned to guide the first few sections of the ribbon up and away from the blade is important for successful sectioning. Ensure your paintbrush has a stable grip on the sections as shown in the gif above. Additionally, the blade may become dull over time due to repeated contact with the paintbrush bristles or forceps. We recommend changing the blade or exposing a new part of the blade by moving the blade holder left or right. If tissue ribbons are still unsuccessful, rehydrate the block until the surface of the tissue is light in color and the paraffin is glowing.

27 **Wrinkled/compressed tissue section**

Cause: Insufficient rehydration or the speed of sectioning may cause artifacts in the tissue sections like wrinkling or compression.



Troubleshooting:

Initially, we recommend rehydrating the tissue block for another 15 min or until the surface of the tissue is light in color and the paraffin is glowing. If the tissue sections are still forming these artifacts, try to slow down sectioning speed to ensure smooth contact between blade and tissue block (figuring out the best speed to section will take time and practice).

Protocol references

Leica Biosystems. *HistoCore*

AUTOCUT Instructions for Use. Version 1.5, RevH.

https://www.leicabiosystems.com/sites/default/files/media_product-download/2022-06/HisotCore_AUTOCUT_IFU_1v5H_en.pdf

10x Genomics. *Xenium In Situ*

Spatial Profiling for FFPE – Tissue Preparation Guide. CG000578, RevE.

<https://www.10xgenomics.com/support/in-situ-gene-expression/documentation/steps/tissue-prep-ffpe/xenium-in-situ-spatial-profiling-for-ffpe-%E2%80%93-tissue-preparation-guide>