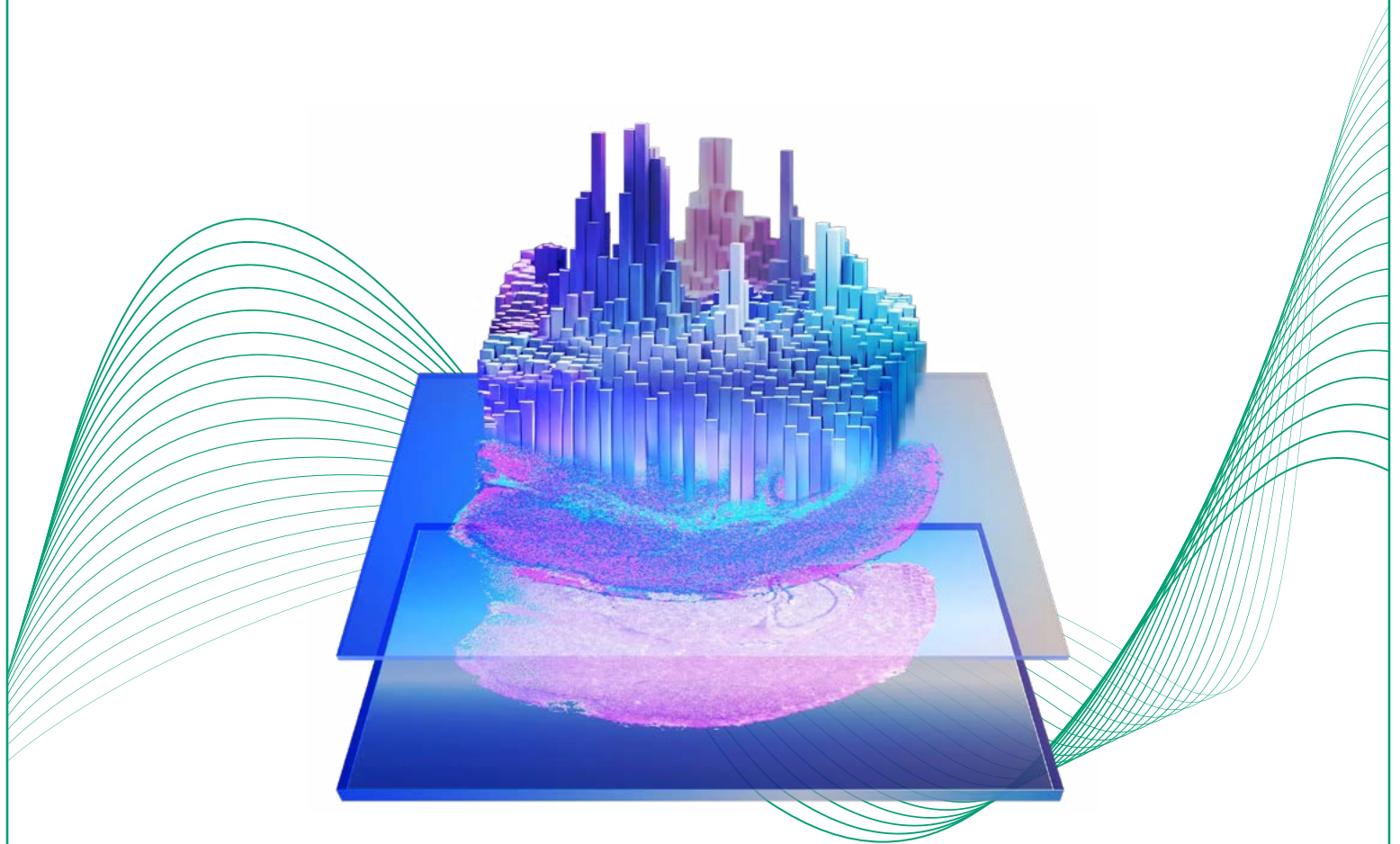


GUIDE

# Visium HD Spatial Profiling Sample Preparation Tips & Tricks



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## VISIUM HD SPATIAL PROFILING SAMPLE PREPARATION TIPS &amp; TRICKS

To obtain the highest quality data, please review the following sample requirements and preparation guidelines prior to submitting samples to GENEWIZ.

## 1. Selecting FFPE Blocks

**When selecting FFPE blocks please consider:**

- The fixation process should be optimized for the tissue types being handled. Refer to [this article](#) for additional guidance.
- Tissues should be immediately fixed in 10% neutral-buffered formalin (NBF) or 4% paraformaldehyde immediately after collecting the tissue.
- Thoroughly dehydrate tissues through ethanol gradients prior to paraffin embedding.
- Store FFPE tissue blocks at 4°C and avoid exposure to direct light to ensure even chilling and preservation of RNA integrity.

## 2. Preparing Unstained FFPE Sections

**We strongly recommend reviewing the [10x Visium HD® FFPE Tissue Preparation Handbook](#) prior to sectioning and following the protocol carefully to prepare high-quality sections. Section 2 contains the instructions for preparing sections. A summary of the critical steps is outlined below.**

- Wipe down all surfaces and work areas with RNaseZap before starting work.
- Carefully wipe a microtome blade with a kimwipe to remove lubricating oils.
- For new blocks, face the block to expose the tissue. For previously sectioned blocks, discard the first few sections.
- Set microtome to 10 µm and collect ~4 sections for tissue sections < 6.5 mm<sup>2</sup> or 1-2 sections for tissue sections > 6.5 mm<sup>2</sup> in a labelled and pre-cooled 1.5 ml microcentrifuge tube. Keep on ice and store long-term at -80°C.
- Adjust the microtome cutting thickness to 3-10 µm. We recommend cutting sections at 5 µm on a calibrated microtome.
- After cutting a ribbon of sections, transfer to a water bath set to 42°C until they are free of folds and wrinkles. Detach the sections using brushes then mount on slides:
  - Use one of the glass slides recommended at the end of this document. Do not use any other slide types to avoid tissue detachment or incompatibility.
  - Prior to placing the section, refer to Visium [CytAssist Accessory Kit Quick Reference Card CG000548](#) and draw an outline of the allowable area on the back of the blank slide to ensure downstream compatibility.
  - **Note:** tissue outside of the 6.5 x 6.5 mm box cannot be processed. If your tissue is larger than 6.5 x 6.5 mm, providing a labelled H&E slide will assist us in selecting the correct area for analysis.

**VISIUM HD SPATIAL PROFILING SAMPLE PREPARATION TIPS & TRICKS**

- After section placement, air-dry for 30 minutes. A fan may be used to assist in drying. Leftover moisture under the tissue may result in tissue detachment.
- Place the slides in a rack and incubate for 3 hours at 42°C.
- Place slides in a slide rack/holder inside a desiccator or a sealed bag containing desiccant. Incubate at room temperature overnight.
- After the overnight incubation, keep at room temperature or 4°C in a sealed bag with desiccant and immediately ship to GENEWIZ from Azenta Life Sciences.

**TIPS AND TRICKS**

- Practice sectioning and correct positioning on nonexperimental blocks.
- Ensure sections are flat and free of any scratches or folds. Scratches and folds may result in tissue loss during the procedure and uninterpretable data.
- If placing multiple sections on a slide, ensure that the paraffin and/or tissue do not overlap.
- Submit slides to GENEWIZ immediately after preparation. Sections that have been stored may not provide optimal results.
- If you need to store slides, it's best to store them sealed in a container with desiccant at 4°C. However, the quality of results on stored slides is tissue and block dependent and results cannot be guaranteed.

### 3. Submitting Archival H&E-Stained Slides

- Use one of the glass slides recommended at the end of this document. Do not use any other slide types to avoid tissue detachment or incompatibility.
- Sections must be flat and free of any scratches or folds. Scratches and folds may result in tissue loss during the procedure and uninterpretable data.
- Only archival slides with glass coverslips and xylene-based mounting mediums, such as Cytoseal, have been tested to be compatible with the Visium HD workflow.
- Archived slides should have been stained, imaged, and stored at room temperature or 4°C. Over time, archived slides may experience RNA degradation; thus, freshly placed FFPE tissue sections are preferred for the Visium HD assay.
- Only archived slides that have been H&E-stained have been tested with the Visium HD workflow.
- For each sample, provide 2-3 H&E-stained slides cut and stained at the same time from the same block. One slide will be used for RNA quality assessment. If this is not possible, use a Sharpie to mark an unimportant area on the underside of the section which can be scraped to assess RNA quality.

### 4. Shipping

- If you're sending FFPE blocks, take preventative measure to eliminate scraping of the block surface during transport.
- Unstained sections should be packed securely in a slide box. Bubble wrap/foam may be inserted to prevent slides from breaking during transport. Place the entire slide box in a sealed bag with a desiccant pack inside.
- Ship FFPE blocks and slides at room temperature or on wet ice.

## 5. Glass Slide Guide

ITEM	PN	IMPORTANT CONSIDERATIONS
Schott Nexterion Slide H - 3D Hydrogel Coated Slides	1800434	10x Genomics recommends these slides for samples with large amounts of connective tissue (like breast or colon) or other tissue liable to detach during processing to minimize risk of tissue detachment.
Epredia Shandon ColorFrost Plus	67-762-14	
Fisherbrand SuperFrost Plus	12-550-15	
Sigma-Aldrich Poly Prep Slides	P0425	
VWR SuperFrost Plus Micro Slide, Premium	48311-703	
Leica X-tra Clipped Corner (White color listed; other colors are acceptable)	3800050CL	
Matsunami Platinum Pro	SPRO-05	
Trajan Series 2 Adhesive Microscope Slide (Yellow color listed; other colors are acceptable)	472042492	
Leica Permaflex Plus (Pink color listed; other colors are also not recommended)	3800019	Slide not recommended. Partial detachment observed with a subset of tissue types tested.
Mutokagaku Star Frost Glass	511511	Slide not recommended. Complete detachment observed with tissue types tested.