TECHNICAL NOTE

Visium Spatial Gene Expression Imaging Guidelines

Introduction

The Visium Spatial Gene Expression Solution measures total mRNA in intact tissue sections and maps where gene activity is occurring. Successful gene expression visualization is highly dependent on good imaging practices. This Technical Note provides hardware recommendations, general image acquisition and analysis guidelines, and examples of images that are suitable for downstream analysis. Individual results may vary depending on the specific imaging system, and/or sample characteristics. Consult the Visium Spatial Gene Expression Reagent Kits Library Preparation User Guide (CG000239) and the Visium Spatial Gene Expression Reagent Kits – Tissue Optimization User Guide (CG000238) for complete protocols.

General Imaging Guidelines

- Proper tissue placement is crucial for successful imaging. Consult the Visium Spatial Protocols – Tissue Preparation Guide for complete information (CG000240).
- Wear a clean pair of gloves when handling slides.
- Ensure slides are clean and dry. Use a laboratory wipe to clean the side without tissue.
- Place slides gently and evenly on the imaging stage.
- Image setting verification and acquisition may be performed with any of the following objectives: 4X (Plan APO λ; NA 0.20), 10X (Plan APO λ; NA 0.45), and 20X (Plan APO λ; NA 0.75).

Any imaging system used for this workflow should have tile scanning functionality for clear imaging of the tissue section on each 6.5 x 6.5 mm Capture Area. Additionally, a computer with sufficient power to handle large images (0.5-5 GB) should be used for image processing.

Imaging System Recommendations

Table 1 shows imaging systems used by 10x Genomics in the development of this protocol. Any equivalent imaging setup can be used as an alternative.

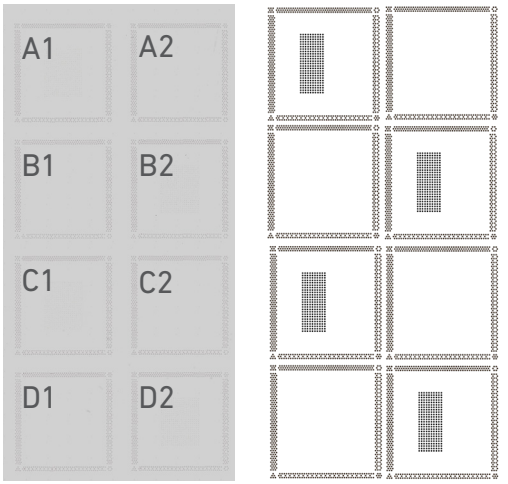
Table 1. Imaging systems used by 10x Genomics.

Supplier	Description
Nikon	Nikon Eclipse Ti2 with brightfield and fluorescence capacity (TRITC and CY5)
Molecular Devices	ImageXpress Nano Automated Cell Imaging System
Brightfield Recommended Configuration	
Color camera (3 x 8 bit, 2424 x 2424 pixel resolution)	
White balancing functionality	
2.18 µm/pixel minimum capture resolution	
Exposure times 2-10 milli sec	
Fluorescence Recommended Configuration	
Light source (or equivalent) with a wavelength range of 380-680 nm	
Monochrome camera (14 bit, 2424 x 2424 pixel resolution)	
TRITC filter cube (Excitation 542/20, Emission 620/52) (Only required for Tissue Optimization and Imaging Test Slides)	
2.18 µm/pixel minimum capture resolution	
Exposure times 100 milli sec-2 sec	

Visium Imaging Test Slide

Slide Information

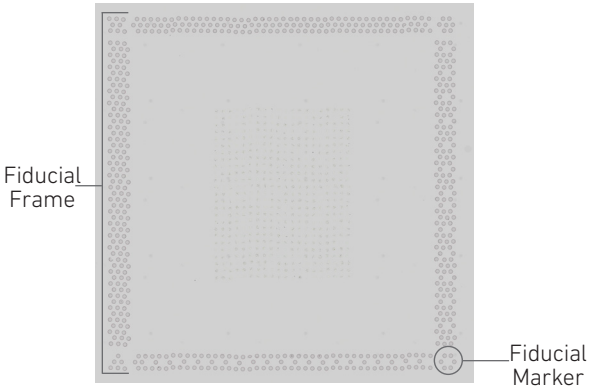
- Use the Visium Imaging Test Slide to verify imaging settings prior to the Visium Spatial Tissue Optimization and Gene Expression workflows.
- Store the unused slide at **room temperature** in its original packaging and keep sealed. DO NOT remove desiccant. Avoid light exposure.
- The Visium Imaging Test Slide has eight areas surrounded by fiducial frames that are visible under brightfield settings. Each fiducial frame has a fiducial marker at each corner.
- Four areas (A1, B2, C1, D2) have fluorescent spots that are detectable with TRITC and Cy5 filter cubes.
- The positioning of fiducial frames matches the Visium Spatial Gene Expression (A1, B1, C1, D1) and Visium Spatial Tissue Optimization slides (all Capture Areas).
- The Visium Imaging Test Slide should be used to create an imaging macro for Visium protocols.



Capture Area labels are illustrative and are not visible under magnification.

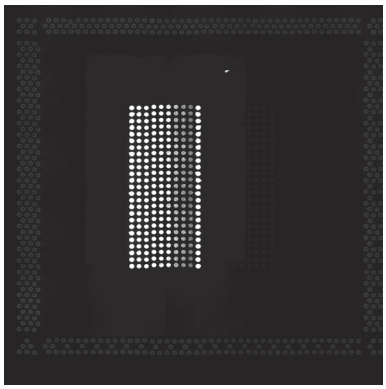
Brightfield Imaging

- Fiducial frames and fiducial markers should be clearly visible and in focus using brightfield settings.
- If fiducial frames are not clearly visible, adjust settings accordingly. Refer to the Imaging Examples.



Fluorescence Imaging

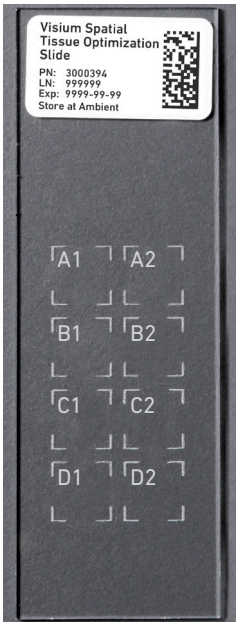
- Fluorescent spots in A1, B2, C1 and D2 should be clearly visible using fluorescence settings.
- Fluorescent spot signal should decrease from left to right, as shown on the image to the right.
- If fluorescent spots are not clearly visible, adjust settings accordingly. Settings may need adjustment depending on tissue.
- After verifying brightfield and fluorescence settings, proceed to the Visium Spatial Tissue Optimization workflow.



Visium Spatial Tissue Optimization Slide

Slide Information

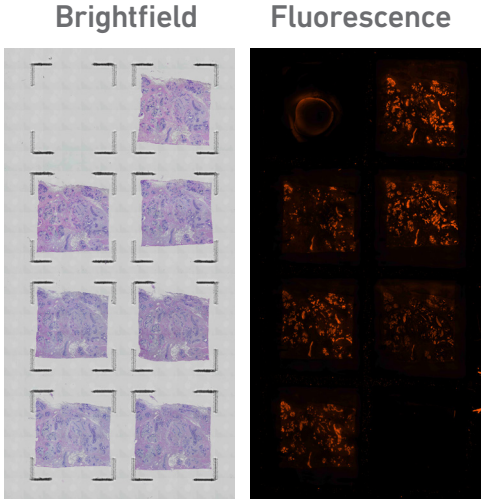
- The Visium Spatial Tissue Optimization slide is used to identify optimal tissue permeabilization conditions. for the Visium Spatial Gene Expression workflow.
- Store unused slides at room temperature in their original container and packaging and keep sealed. DO NOT remove desiccant.
- The Visium Spatial Tissue Optimization Slide has eight 8 x 8mm Capture Areas surrounded by etched frames.
- Each Capture Area contains oligonucleotides for mRNA capture. Each oligonucleotide allows for the production of fluorescent cDNA from poly-adenylated mRNA using fluorescent nucleotides.
- Fluorescence signal is used as a proxy for permeabilization efficiency. Fluorescence imaging is not required for the Visium Spatial Gene Expression workflow.
- Consult the Visium Spatial Tissue Optimization User Guide for complete information on the tissue optimization workflow (CG000238).



Capture Area labels are illustrative and are not visible under magnification.

Imaging Guidelines

- During brightfield and fluorescence imaging, image all eight Capture Areas at once without using autoexposure.
- Compare fluorescence images with brightfield images to ensure that a lack of signal is due to insufficient permeabilization, not missing tissue.
- Select the permeabilization condition that results in the highest fluorescence signal with the lowest signal diffusion. See next page for examples.
- If the signal is the same at two time points, the longer permeabilization time is considered optimal.



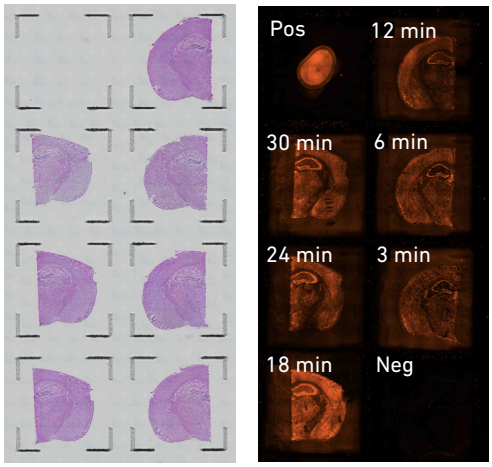
Visium Spatial Tissue Optimization Examples

General Settings

- All examples use 10 µm tissue sections.
- All tissue sections were imaged using a Nikon Eclipse Ti2 microscope with the following settings: 10x NA 0.45 objective, 0.73µm/pixel capture resolution, TRITC filter cube, 75% Sola pad.

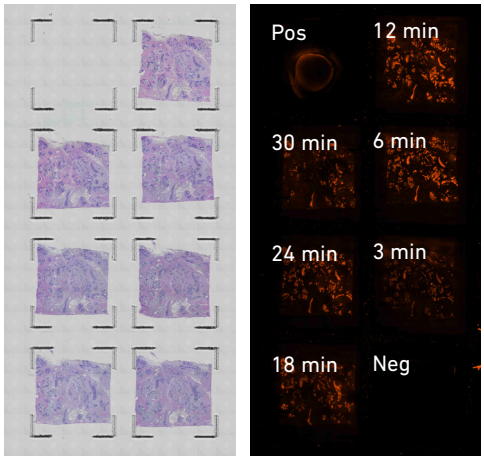
Mouse Brain

- Exposure: 200 ms.
- Time selected: 18 min.
- The negative control shows no fluorescence signal, while the positive control shows a strong signal.
- Low signal after 3-12 min of permeabilization suggest insufficient permeabilization.



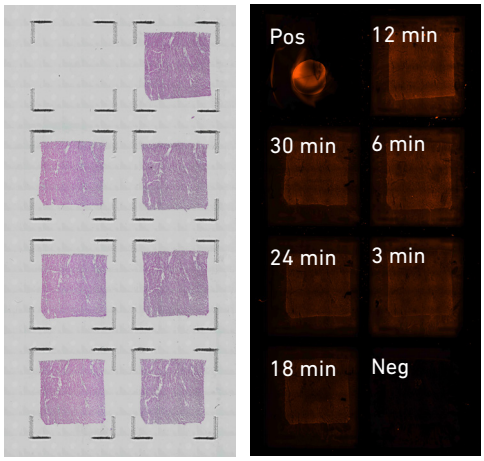
Human Breast

- Exposure: 200 ms.
- Time selected: 12 min.
- Assuming uniform reagent coverage, variation in fluorescence signal within the same tissue section is normal and reflects biological variability in transcription.
- Fluorescence signal is similar between 12 min and 6 min, therefore the longer time was selected for the Visium Spatial Gene Expression workflow.



Human Heart

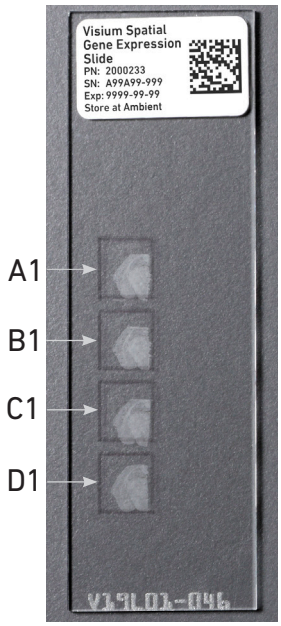
- Exposure: 300 ms.
- Time selected: 12 min.
- Although fluorescence signal is dim, this permeabilization time course is considered successful. Dim fluorescence signal is expected due to low RNA content.



Visium Spatial Gene Expression Slide

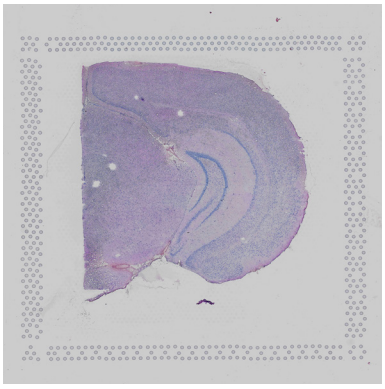
Slide Information

- The Visium Spatial Gene Expression slide is used to generate libraries from frozen tissue sections.
- Store unused slides at **room temperature** in their original container and packaging and keep sealed. DO NOT remove desiccant.
- Store slides with tissue sections in a sealed container at -80°C for up to a week.
- The Visium Spatial Gene Expression Slide has four Capture Areas surrounded by fiducial frames.
- Each Capture Area has ~5,000 unique gene expression spots.
- Fiducial frames are used by software to align the image.
- Consult the Visium Spatial Gene Expression Reagent Kit User Guide for complete protocol (CG000239).

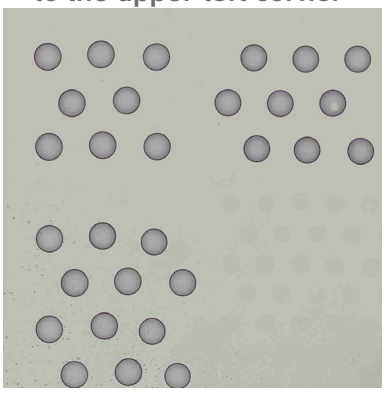


Imaging Guidelines

- Image each capture area individually, as shown on the right. Minimize imaging of adjacent Capture Areas.
- Imaging area should be ~1-2 mm beyond the fiducial frame for optimal imaging alignment.
- After image acquisition, stitch image tiles together with the microscope's native software or third party software such as ImageJ.
- Each stitched image should correspond to one Capture Area.
- Ensure that images are oriented such that the pattern on the photo to the right is on the upper left corner.
- Export stitched images as a 24-bit color or 16-bit monochrome tiff (preferred) or jpeg image.
- Name the file using both the serial slide number (i.e. V19L29-033) and Capture Area identifier in a manner compatible with the user's desktop and cluster environments.
- For information on image analysis and tissue alignment, refer to the 10x Genomics Support Website.

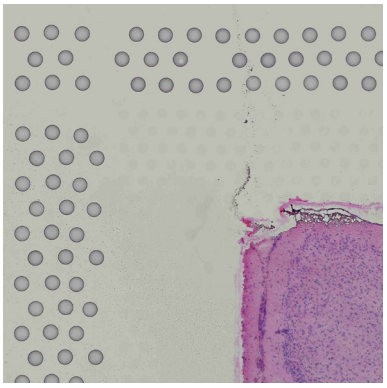
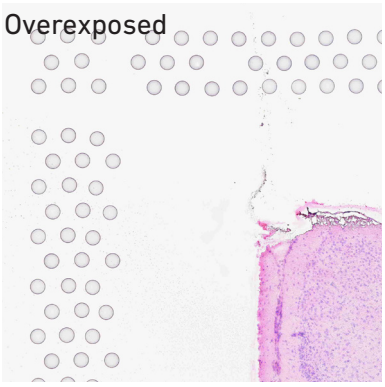
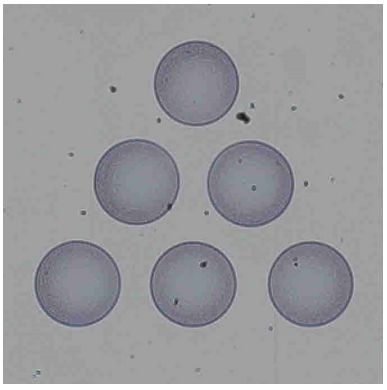
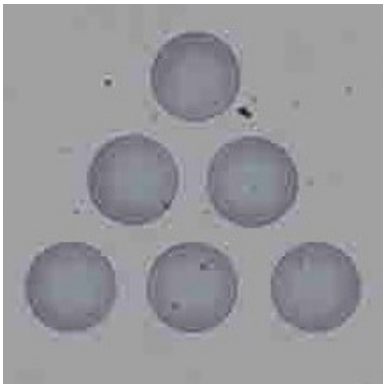


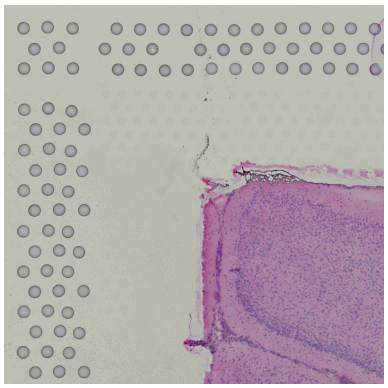
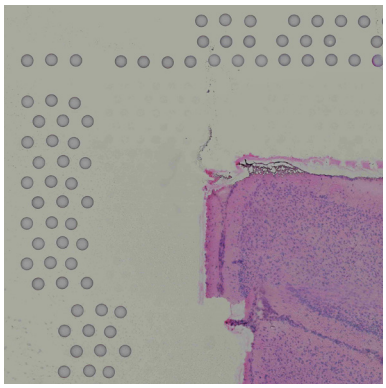
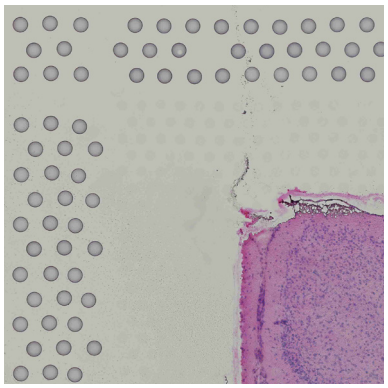
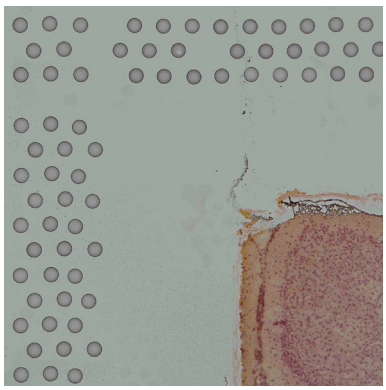
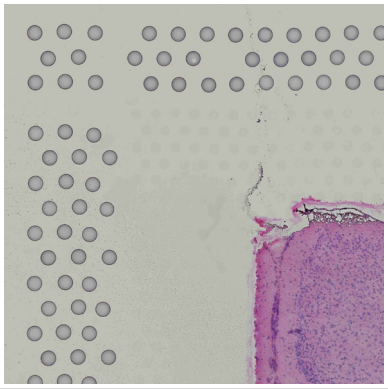
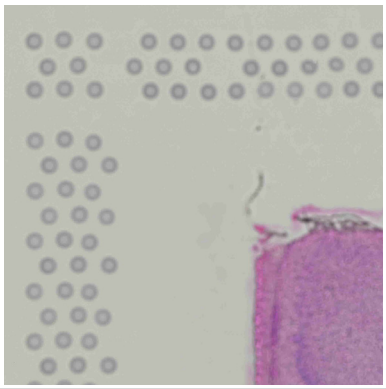
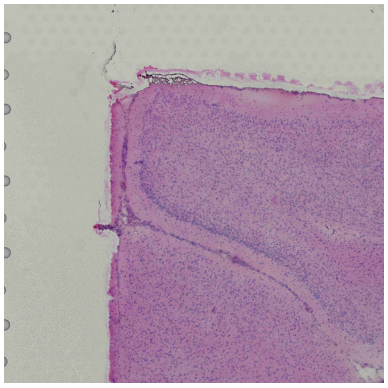
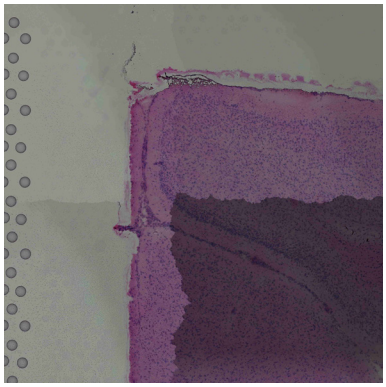
Orient this fiducial marker to the upper left corner



Imaging Examples

The following imaging artifacts may cause image analysis failure. Ensure that optimal imaging settings are verified prior to beginning the Visium Spatial Gene Expression workflow.

CONCEPT	CORRECT	INCORRECT
Image Exposure		 
Capture Resolution	 Capture resolution: 5.5 µm/pixel	 Capture resolution: 0.73 µm/pixel

CONCEPT	CORRECT	INCORRECT
Stitching		
White Balancing		
Focus		
Shade Correction		

References

- Visium Spatial Gene Expression Reagents Kit - Tissue Optimization User Guide (Document CG000238)
- Visium Spatial Gene Expression Reagent Kits User Guide (Document CG000239)
- Visium Spatial Protocols - Tissue Preparation Guide Demonstrated Protocol (Document CG000240)

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