Visium HD Spatial Applications Imaging Guidelines

Introduction

The Visium HD platform is designed to analyze tissue sections at single cell scale. Before running a Visium HD assay, a microscope image is captured. An image taken by the Visium CytAssist instrument is used to map gene expression data back to the microscope image. This Technical Note provides hardware recommendations, general image acquisition and analysis guidelines, and examples of images that are suitable for downstream analysis with Space Ranger and Loupe Browser.

Individual results may vary depending on the specific imaging system, and/or sample characteristics.

This Technical Note applies to all Visium HD assays.

1. Slide Preparation

- Wear a clean pair of gloves when handling slides.
- DO NOT touch or wipe the side of the slide with tissue.
- Ensure slides are clean. If necessary, use a laboratory wipe or compressed air to clean the back (side without tissue) of the slide before imaging.
- Place slides gently and evenly on the imaging stage.
- For optimal quality, mount slides with a coverslip. Use a coverslip appropriate for the chosen objective. Most objectives are corrected for #1.5 (0.17 mm) coverslips. Objectives with a correction collar (20X or higher) can accommodate other types of coverslips.

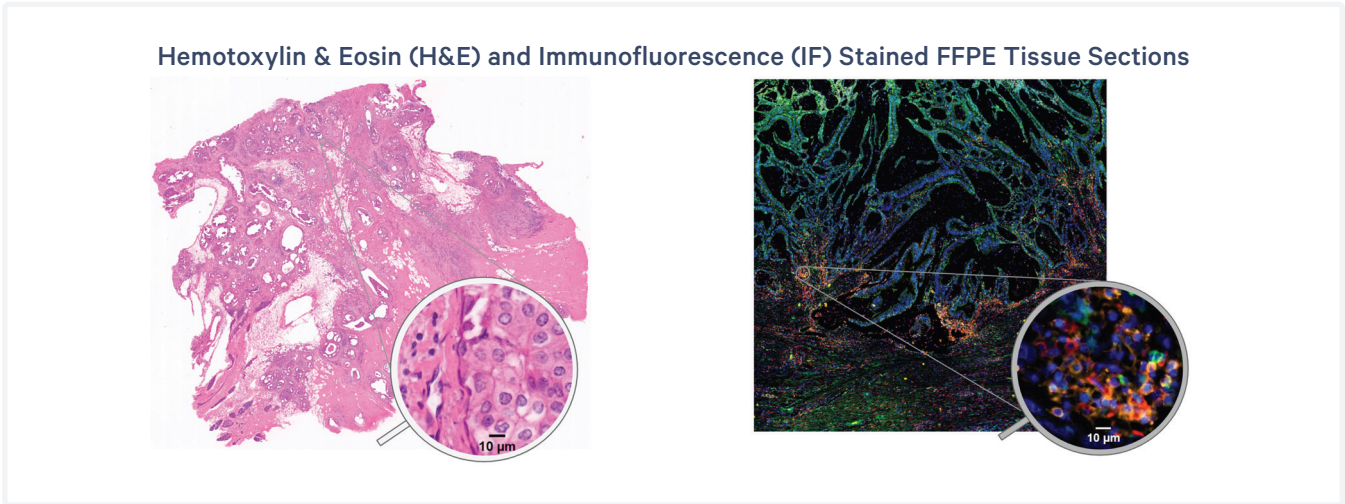


Figure 1. H&E stained human breast cancer (left) and IF stained human colon (right) tissue. Circles within each image show a zoomed-in view of the tissue. The images were acquired using an Olympus VS200 slide scanner equipped with 20x/0.8 NA objective lens and color camera with 3.45 µm pixel size (H&E image) or Hamamatsu Orca Fusion BT camera with 6.5 m pixel size (IF image).

- Fresh tissue sections should be loosely mounted with a nonpermanent mounting medium, such as glycerol for H&E samples or SlowFade Diamond for IF samples. The coverslip will be removed before the assay workflow.
- Remove excess mounting media before imaging, as mounting media overflow may damage the imaging system. Inverted systems are especially prone to this type of damage. The amount of mounting media may be reduced depending on tissue size.
- If a coverslip is not used for an H&E stained sample, blackening of hematoxylin may occur.
- Fluorescently stained samples should be mounted with an appropriate antifade mounting media that will minimize photo bleaching during imaging.
- Coverslip application and removal guidance, for both archived and fresh tissue sections, are provided in documents found in the References section.

2. Imaging System Requirements & Recommendations

When visualizing spatial gene expression data, the resolution of the tissue image should match the single cell scale offered by Visium HD. The image resolution is a combination of objective resolution (dependent on numerical aperture (NA)), objective magnification, and camera pixel size. The optimal image resolution will vary depending on cell size, the contrast of stained features, and the desired level of detail. Follow manufacturer-recommended imaging settings to obtain the best resolution.

The size of objects that are clearly distinguishable in an image is typically 5–10 times the image resolution. 10x Genomics recommends a resolution between 0.3–1.0 µm for color BF images and 0.3–2.0 µm for IF images. Though images within these resolution recommendations are ideal, images with reduced resolution are also compatible with Space Ranger and Loupe Browser. These images can be used when a lower level of detail is acceptable for downstream analysis and interpreting Visium HD data.

Table 1 below shows correlation of imaging system configuration (low to high resolution) with cellular features that are distinguishable as well as the impact on image file size. Example images are provided below.

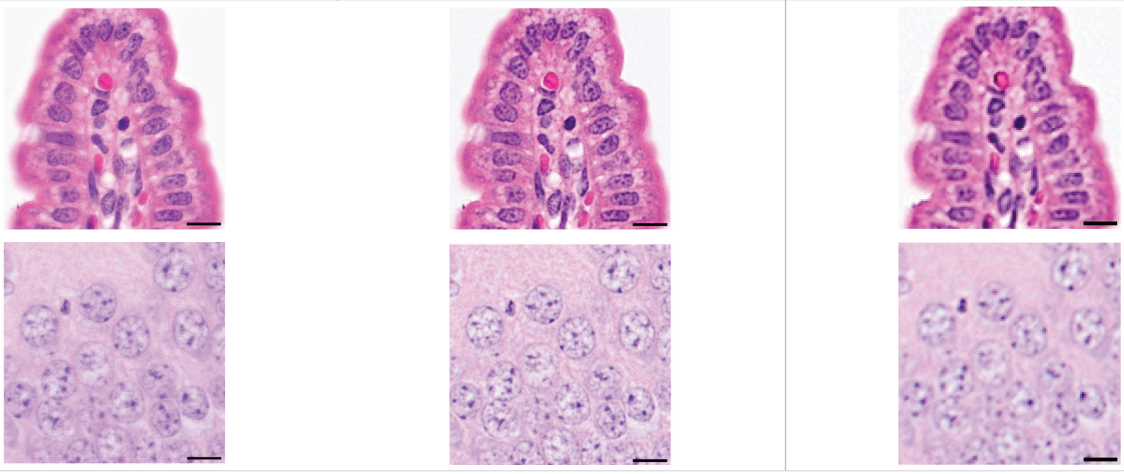
Example image systems tested by 10x Genomics are provided in Table 2. Any equivalent imaging system can be used as an alternative and is dependent on user needs and applications. Automated acquisition and stitching of multiple fields of view (FOVs) is required when imaging with an imaging system.

	Objective Magnification / NA	Objective resolution (µm, 0.61*λ / NA)	Image Pixel Size (µm)	Distinguished Features	File size 10x10 mm tissue, H&E 24 bit / IF 3x16 bit	Compatible file format
Recommended for IF	10X / 0.3	1.1	0.65	Large cells, tissue compartments with 1-10 cells (10 µm)	1 GB / 1.5 GB	TIFF, JPEG
Recommended for BF and IF	20X / 0.5	0.67	0.33	Single cells, nuclei (5 µm)	4 GB/ 6 GB	BigTIFF
	20X / 0.8	0.42	0.33	Single cells, nuclei (5 µm)	4 GB / 6 GB	BigTIFF
	40X / 0.75	0.45	0.16	Nuclei, nucleoli (3 µm)	4 GB / 6 GB	
	40X / 0.95	0.35	0.16	Nuclei, nucleoli, mitochondria (2 µm)	16 GB / 23 GB	BigTIFF
Not Recommended	40X / 1.4*	0.24	0.16	Nuclei, nucleoli, mitochondria (2 µm)	16 GB / 23 GB	BigTIFF
	60X / 1.49*	0.22	0.11	Mitochondria, large lysosomes; limit of optical resolution (1 µm)	26 GB / 50 GB	BigTIFF

Table 1. Image resolution and file size obtained with example objectives. Pixel size was calculated for a camera with a 6.5 µm pixel. *Objective lenses with higher magnification and NA may be used, but have important considerations (See Image Resolution).

Resolution – Brightfield

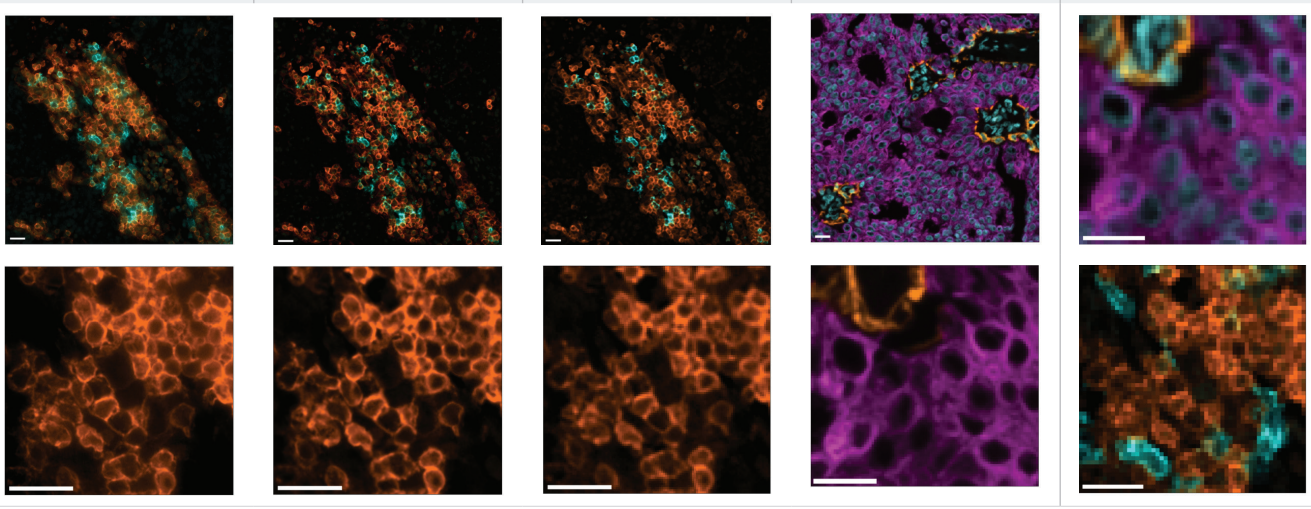
Image Resolution < 1 µm (recommended)		Image Resolution > 1µm (not recommended)
Subcellular < 5 µm	**Small Cells, Nuclei 5–10 µm**	**Pixel Size > 0.45 µm**
Image Resolution 0.35 µm 40x/0.95 NA (res 0.35 µm) Image Pixel 0.14 µm	Image Resolution 0.63 µm 20x/0.8 NA (res 0.42 µm) Image Pixel 0.27 µm	Image Resolution 1.26 µm 10x/0.45 NA (res 0.75 µm) Image Pixel 0.55 µm



Images were acquired with different microscopes.
Sample: H&E-stained stained villus of mouse small intestine (top), H&E-stained dentate gyrus (bottom), Scale bars = 10 µm

Resolution – IF

Image Resolution < 2 µm (recommended)				Image Resolution > 2 µm (not recommended)
Subcellular < 5 µm	**Small Cells, Nuclei 5–10 µm**	**Single Cells, Large Nuclei 10–15 µm**	**Large Single Cells > 15 µm**	**Pixel Size < 0.8 µm**
Image Resolution 0.37 µm 40x/0.95 NA (res 0.35 µm) Image Pixel 0.16 µm	Image Resolution 0.75 µm 20x/0.8 NA (res 0.42 µm) Image Pixel 0.33 µm	Image Resolution 1.5 µm 10x/0.3 NA (res 1.1 µm) Image Pixel 0.65 µm	Image Resolution 1.85 µm 10x/0.45 NA (res 0.75 µm) Image Pixel 0.8 µm	Image Resolution 3 µm 10x/0.45 NA (res 0.75 µm) Image Pixel 1.3 µm



Images were acquired with different microscopes.
Sample: IF-stained human lung cancer: CD20 (cyan) and CD45R0 (orange). IF-stained human breast cancer: nuclei (cyan), alpha smooth muscle (orange), KRT18 (magenta). Scale bars = 20 µm

Image resolution

The effective resolution of an image is given by the greater of the objective's resolution or the size of resolvable detail based on the image pixel size (approximately 2.3 times the image pixel size).

The objective resolution is calculated as 0.61*λ/NA, where NA is the objective's numerical aperture and λ is the wavelength of the illumination light (for the visible spectrum, the average λ is 550 nm). Single cells can be distinguished starting at NA 0.3 or higher (objective resolution < 1.2 µm), while objectives with NA in the range of 0.75–1.40 can resolve subcellular compartments such as nuclei, mitochondria, and lysosomes (objective resolution 0.24–0.45 µm). 10x Genomics recommends using objectives with NA between 0.40–0.95 for color BF imaging and 0.30–0.95 for IF imaging.

The image pixel size can limit the image resolution when it is higher than the objective resolution. To preserve the resolution provided by the objective, the image pixel size should be equal to or smaller than the objective resolution divided by 2.3. The image pixel size is typically displayed in the imaging software and saved in the image metadata. It can be calculated by dividing the camera pixel size by the objective's magnification, assuming that the optical path does not introduce an adjustment to magnification. 10x Genomics recommends an image pixel size in the range of 0.10–0.45 µm for color BF imaging or 0.1–0.8 µm for IF imaging.

Objective lenses

Air objectives with magnification of 10x, 20x, and 40x and NA 0.30–0.95 are recommended for image acquisition. Air and immersion (water, silicon, oil, etc.) objectives with higher magnifications (60x or 100x) or higher NA (1.0 or higher) may also be used, but have caveats described in the bullets below. DO NOT use immersion media on air objectives. Objectives with 10x magnification and NA 0.3–0.5 can be used when imaging tissues with larger cells, when imaging samples with high contrast (e.g. IF images), or when the level of detail is sufficient for data interpretation. To fully utilize the resolution offered by the objective, we recommend imaging through the coverslip. This is especially important when using an objective with magnification 20x or higher.

If using a high magnification objective (40x or higher) or a high NA (1.0 or higher), consider the following:

- The depth of focus (DF) will be < 1 µm and as low as 0.3 µm. The depth of focus is the sample thickness that appears in focus within one image plane. The higher the objective NA, the lower the DF. In a 5 µm tissue section, some structures will look out of focus when one image plane is acquired. A z-stack that covers the tissue thickness with a step size of 0.3–1 µm between image planes is recommended. The z-stack needs to be processed to obtain a single image projection.
- The FOV will be small, typically less than 0.2 x 0.2 mm. To create an image that contains the entire tissue section, hundreds of FOVs will need to be acquired and stitched. This increases the probability for stitching errors, which can lead to misregistration with corresponding gene expression data.
- Acquiring hundreds of FOVs, each with a z-stack, will result in extended time of acquisition and processing (more than 1 h on some systems).
- The resulting images will be large (> 1,000 MP) with file sizes most often > 5 GB and may reach 100 GB. Working with these images requires a computer with appropriate memory resources and specialized software to upload, visualize, and export images as BigTIFFs. The time required to handle large files can be significant, on the order of tens of minutes per image.

Tested Imaging Systems & Specifications

Recommended			
Supplier	**Description**	**Acquisition Method**	**Features & Considerations**
Evident/Olympus	VS200	BF/IF	Automatic slide scanner; can batch many slides.
Hamamatsu	NanoZoomer S60	BF	
Motic	EasyScan One	BF	Automatic slide scanner; can image <5 slides at a time.
Mikroscan	Mikroscan SL5	BF	Automatic slide scanner; can image 1-2 slides at a time.
Nikon	Nikon Eclipse Ti2	BF/IF	Manual customization of system settings (autofocus, stitching methods, camera properties etc.) and acquisition modes (large image vs individual FOVs).
Zeiss	Axio Imager.Z2	BF/IF	Higher user expertise requirements compared to automatic scanners.
Not Recommended			
Supplier	**Description**	**Acquisition Method**	**Features & Considerations**
Thermo Fisher Scientific	EVOS M7000	BF/IF	Not recommended due to: • Image stitching errors • Image downsizing • Autofocus issues Refer to Image Compression and Downsizing within Troubleshooting and contact vendor for additional support
Microscope Features (Any equivalent system with the listed features may be used for imaging)			
Objective Lenses	• Magnification of 10X, 20X, 40X • Numerical aperture (NA) between 0.3–0.95 • Plan APO correction • Air (dry) objectives		

Cameras	• Brightfield: color camera
	• Fluorescence: monochromatic camera
	• Example cameras
	  • iDS VS-264C color camera (3.45 µm pixel size, 3 x 8 bit)
	  • Zeiss Axiocam 712 (3.45 µm pixel size, 3 x 8 bit)
	  • Nikon Ri2 (7.2 µm pixel size, 3 x 8 bit)
	  • Hamamatsu Orca Fusion BT sCMOS (6.5 µm pixel size, 16 bit)
	  • Nikon Qi2 (7.2 µm pixel size, 16 bit)		

Image Acquisition	• If possible, calibrate the microscope and cameras (alignment, white balance, flatness of field of view) according to manufacturer specifications • If possible, adjust illumination settings (light source power, camera gain, and exposure) to obtain a bright and clear image without saturation • BF: NA 0.4–0.95, image pixel size between 0.1–0.45 µm • IF: NA 0.3–0.95, image pixel size between 0.1–0.8 µm		
Scanning Functionality	• Automated stage • Ability to program acquisition of multiple tiles / fields of view • Automated stitching of acquired tiles into a large image. This should not be performed manually.		
Software and File Formats			
Computer	Sufficient memory to handle large images (1–100 GB).		
Image Format	Save/Export images as TIFF (images < 4 GB), BigTIFF, OME-TIFF, or JPEG (images < 2 GB)		
Software	Microscope native software, QuPath, FIJI/ImageJ (compatible with images < 2 GB)		

Table 2. Summarized table of Visium HD tested imaging systems and imaging recommendations.

Cameras

A color camera is required for H&E stained tissue and a monochromatic capable camera/detector for IF stained tissue sections.

Camera pixel size influences image resolution and quality. Most available cameras have pixel sizes 1.5–16 µm. Cameras with small pixel sizes typically have less sensitivity, higher noise, and require more careful adjustment of illumination conditions to increase signal and decrease noise, especially when imaging fluorescently-labeled tissue. The following guidelines describe objective and camera combinations that preserve 0.3–2.0 µm system resolution.

Objective magnification	Camera pixel size
10x	1.5–8.5 µm
20x	3–17 µm
40x	5.5–34.5 µm
60x	8–52 µm

3. Brightfield Imaging Guidelines

Perform Hematoxylin and Eosin (H&E) staining and imaging before the Visium HD workflow. Consult the documentation listed in the References section.

H&E stained tissue sections are imaged with a color camera. Slide scanners and some microscopes are automated for optimal imaging. For microscopes that require manual setting adjustments:

- Adjust the microscope for Köhler illumination: This adjustment of the condenser focus and position provides uniform and strong illumination of the tissue and ensures good contrast and clarity. See manufacturer directions for performing this adjustment.
- Adjust illumination power and exposure time to obtain a bright image without saturation and clear tissue morphology. Poorly exposed images have low contrast, loss of data, and compromised resolution which cannot be restored after.
- Adjust the color balance on the camera by performing a white balance on an empty area.
- Include shading correction when stitching individual FOVs together using the microscope's native software.

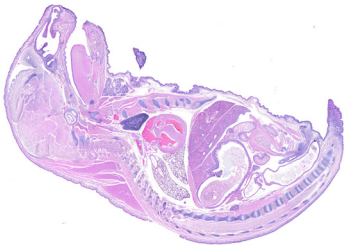
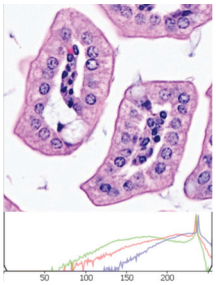
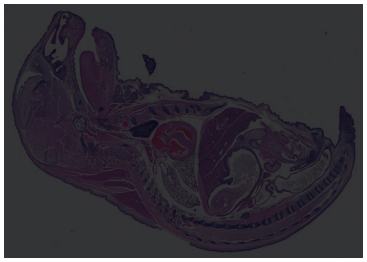
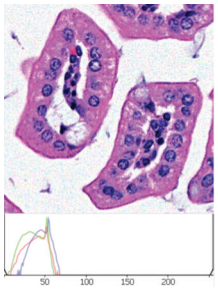
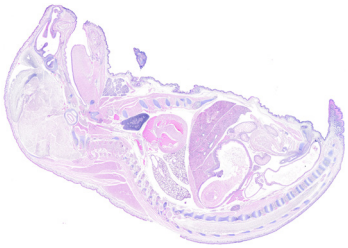
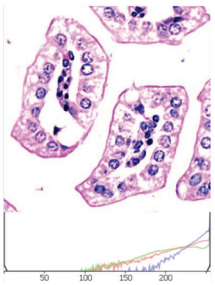
4. Fluorescence Imaging Guidelines

Immunofluorescence staining and imaging should be performed before the Visium HD workflow and is only required if alignment of specific protein staining with gene expression is desired. Nuclear staining (eg. DAPI) is necessary when performing immufluorescence staining and is essential for the analysis pipeline.

Consult the documentation listed in the References section for full staining protocol, autofluorescence quenching, and antibody optimization.

Fluorescence imaging is performed with a mono-chromatic camera/detector. For microscopes that require manual setting adjustments, verify the following:

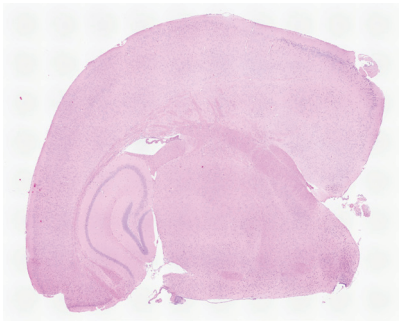
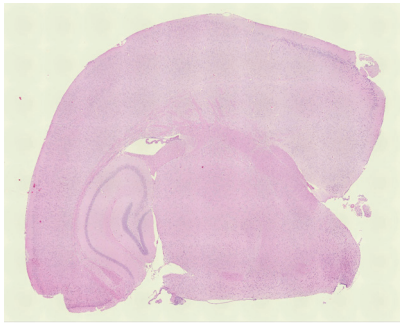
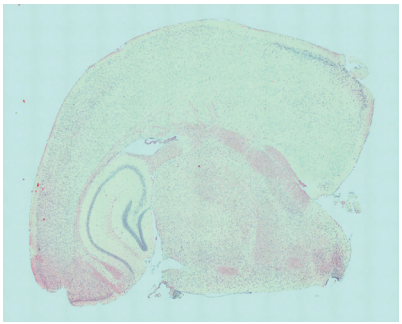
- Adjust the illumination power, detector gain (if available), and exposure time to obtain a clear and nonsaturated image. Poorly exposed images have low contrast, loss of data, and compromised resolution, which cannot be restored after acquisition.
- Acquire images with correction for the field of view flatness. Without this correction, the fluorescence intensity is typically lower at the edge of the field of view compared to its center.
- When imaging multiple fluorescent stains, check the images for any noticeable shift between the channels taken at different wavelengths. If large shifts that could impair proper single cell identification are found, perform system calibration for wavelength registration or determine the shift between channels using multispectral beads. The values determined from multispectral bead images can be used to manually correct the shift between channels, typically using the imaging software or using a third-party software like FIJI/ImageJ or QuPath.
- Stitch individual FOVs without shading correction that could alter the acquired fluorescence levels. Some shading artifacts might be visible at the junction between individual FOVs, especially when the images have low signal, high autofluorescence, or if the flatness of field of view was not corrected. When comparing the fluorescence intensity between images or within one image is not needed, stitching can be done with shading correction to correct for these artifacts.

Image Exposure		
Correct	**Incorrect**	
 	 	 
Appropriate exposure provides good brightness and contrast. Image look up table (LUT) shows the histogram of pixel intensities that spans most of the dynamic range of the camera (0-256) without saturated pixels.	Underexposed images have low brightness (low pixel intensity values) and low contrast (low image dynamic range) which results in inaccurate color, loss of detail, and compromised resolution, even when the brightness is adjusted postimaging (zoomed-in area). The LUT shows that the histogram of pixel intensities spans the lower quarter (0-60) of the full camera range.	Overexposed images have high brightness with saturated pixels (white or lighter color) and low contrast that results in inaccurate color, loss of data, and compromised resolution, even when the brightness is adjusted postimaging (zoomed-in area). The LUT shows that the histogram of pixel intensities is clipped at the high end, indicating that a large percentage of pixels are saturated, and spans the upper half of the camera range (100-256).

Imaging system : Olympus VS200, iDS VS-264C color camera, 20x/0.8 NA objective lens

Sample: H&E-stained, postnatal day 1 mouse pup section. Zoomed-in H&E image is a zoomed-in section of the larger H&E image.

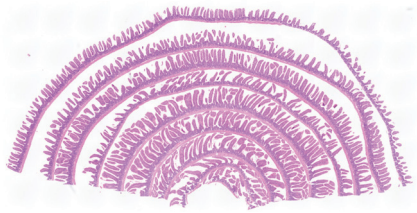
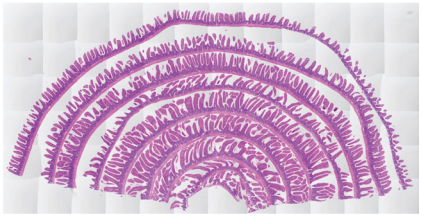
White Balance

Correct	Incorrect
	 
Good color balance: Illumination halogen lamp set to high power (more similar to daylight) together with white balance performed on an empty area produces an image with good color hue and contrast, accurate to the stained specimen.	Off balance: When illumination is set to a low power (more yellow light) and white balance is not performed, images will have a yellow tint (left). Performing white balance on an area with stained tissue will also result in images with false colors (right). The compromised color and contrast will make it more difficult identifying tissue structures and properly annotating the images that depend on the various shades of pink of eosin.

Imaging System: Zeiss Axio Imager.Z2, Axiocam 712 color camera, 20x/0.8 NA objective lens

Sample: H&E-stained mouse brain tissue section

Shading

Correct	Incorrect
	
Applying shading correction when stitching many FOVs into a large image will correct for artifacts due to nonuniformities in the field of view. Proper alignment and setting of the illumination path can also reduce shading artifacts.	When the field of view is nonuniformly illuminated, shading artifacts may be visible when stitching many FOVs without correction. The image to the left has the same FOVs with proper shading correction.

Imaging System: Nikon Ti2 microscope, DS-Ri2 color camera, 20x/0.75 NA objective lens

Sample: H&E-stained mouse small intestine

5. Image Format Requirements & File Size Considerations

Use a computer system with sufficient resources to handle large images (1–100 GB). Preinstall the computer system with the most recent version of Loupe Browser to support manual alignment and tissue selection for IF and H&E stained tissues. To run Space Ranger, 10x Genomics recommends a computer with 64 GB of RAM or better. A computer system with this amount of RAM can handle a maximum image size of 4.5 gigapixels, or ~67,000 x ~67,000 pixels.

Space Ranger v2.1 and newer, and Loupe Browser v6.5 and newer, accept TIFF (.tif, .tiff), BigTIFF (.tf2, .tf8, .btf), qpTIFF (.qptiff) or JPEG (.jpg, .jpeg) image formats. Space Ranger and Loupe Browser support pyramid TIFF or multiple single TIFF/bigTIFF images that contain multiple resolutions. A multipage TIFF/BigTIFF file can contain up to six total pages/images with the same bit depth.

Individual channel images from fluorescently labeled samples can be submitted separately with up to six total files from the same tissue section. Ensure that each monochrome image has been acquired at the same magnification, has the same bit depth, dimension, orientation, and file format.

When the acquisition software does not have the option to save or convert the images in one of the compatible format types, use third-party software to make the conversion, like QuPath or FIJI/ImageJ. FIJI/ImageJ can open files < 2 GB. For larger images, QuPath can be used to process, save, and export images. Some file formats have size limitations. TIFF can hold up to 4 GB of data, while JPEG can hold up to 2 GB of data. For large images, the files should be saved or exported as BigTIFF.

6. CytAssist Imaging Guidelines

- The Visium CytAssist instrument captures a single FOV image of the tissue section, which can be automatically registered to a microscope H&E or IF image of the same tissue section (if provided) in the Space Ranger analysis pipeline or can be registered using Loupe Browser 6.2 or later.
- Though only the area of interest needs to be imaged, imaging the entire tissue is preferred.
- If the tissue section is larger than the Capture Area, the area of interest may highlighted by annotating the back of the Tissue Slide using a marker.
- Any annotations on the Tissue Slide must be removed prior to loading onto the CytAssist. Failure to remove the annotations on the back of the tissue slide can lead to improper tissue detection, causing automatic tissue registration to fail which will then require manual tissue registration on Loupe browser.

Troubleshooting

Image Compression and Downsizing

Some imaging systems will impose constraints on file size, resulting in compression or downsizing of large images. This reduces the final resolution of the image. If the resolution is unexpectedly low, verify the image pixel size. If pixel size is larger than the camera pixel size divided by objective magnification, contact the manufacturer to determine whether the optical path is modified or if image downsizing has been applied to acquired images.

If the image is downsized, consider:

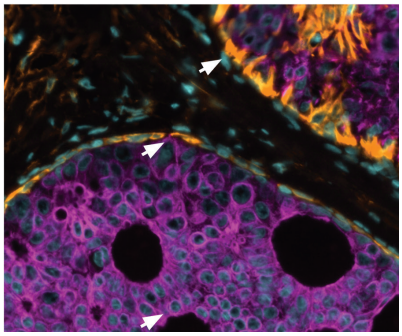
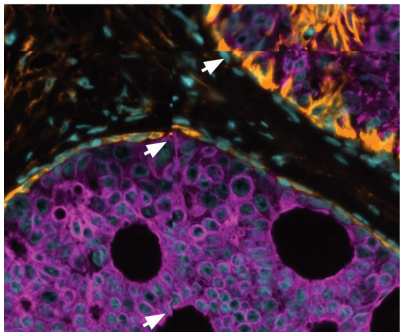
- Imaging a smaller tissue area as well ensuring max file size during stitching
- Saving individual FOVs and stitch with an external software

Image Stitching

When imaging a large tissue area, multiple FOVs are stitched together and we recommend using a stitching algorithm that will account for inaccuracies due to stage properties. However, stitching algorithms have limitations and can introduce artifacts.

Stitching errors can be more pronounced when using higher magnification objectives (i.e more number of FOVs), for tissues that have similar repetitive features as well as the performance of the stitching algorithm used by the imaging software. If error rates are very high consider:

- Using a lower magnification objective which reduces the number of FOVs and increases the probability of unique features that can be stitched better.
- Turning off the stitching mode and visually evaluate if these images have minimal errors compared to the stitched ones.

Stitching	
Correct	**Incorrect**
	
Stitching with registration: When combining multiple FOVs in a large image, stitching with registration will correct errors in tissue alignment between adjacent FOVs due to stage inaccuracies (compare white arrows with the nonstitched image).	No stitching: When multiple FOVs are combined in a large image relying only on the stage coordinates without image registration, tissue mismatch may be visible at the border between adjacent FOVs (white arrows), typically due to inaccurate stage movement. This will result in inaccurate localization of cells within the tissue.

Imaging system : Zeiss Axio Imager.Z2, Axiocam 712 color camera, 20x/0.8 NA objective lens

Sample: IF-stained human breast cancer: nuclei (cyan), alpha smooth muscle (orange), KRT18 (magenta)

Focus Issues

When imaging a large area that will span multiple FOVs, a focus map has to be created so that each FOV will be in focus in the final image. The change in exact focus across a large area is due to slight tilt of the microscope stage or due to slide placement on the stage. Within a single FOV some areas might appear blurry when the tissue is uneven and the thickness is higher than the depth of field of the objective lens. This effect will also be present when using a microscopy technique that creates an optical section for each acquired image (e.g. confocal, two-photon laser imaging, using the Apotome on a Zeiss widefield microscope, etc).

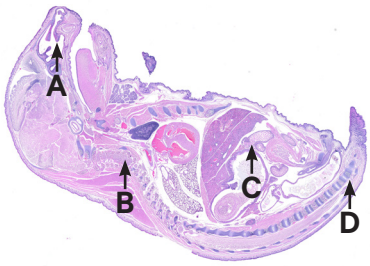
When focus is uneven within a single FOV:

- The microscope stage is not leveled properly. Typically one side of the FOV is out of focus.
  - » Level your stage and/or take a z stack followed by creating a single image projection of the acquired z stacks.
- Tissue surface is uneven and some regions are thicker than the objective's depth of focus.
  - » Consider using lower NA which increases the depth of focus and/or take a z stack followed by creating a single image projection of the acquired z stacks.
- The microscopy technique creates an optical section
  - » Consider imaging on a widefield microscope or change imaging setting to maximize optical section (e.g. open the pinhole to its maximum size when using confocal imaging). Using an objective lens with lower NA will also increase the thickness of the optical section.

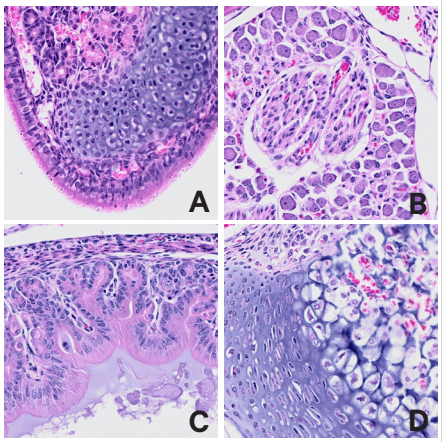
When the uneven focus is observed across the large stitched image

- Likely issues with the creation of the Focus map
  - » Manually increase the number of focus points to improve the focus map or set up autofocus for every FOV. Adjust autofocus settings if the system allows. For additional support, contact the microscope vendor.

Focus

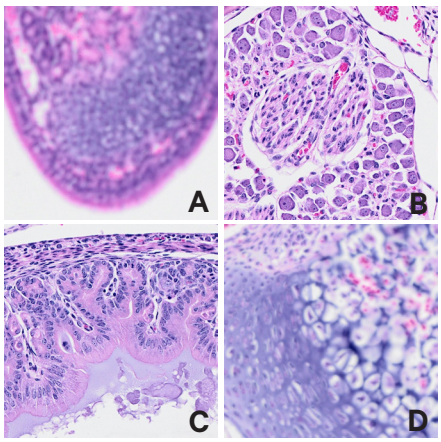


Correct



Focus surface created from several focus points across the sample.

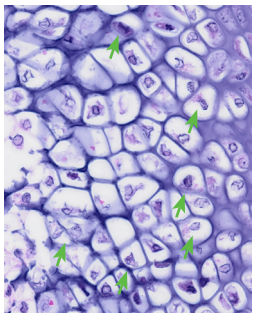
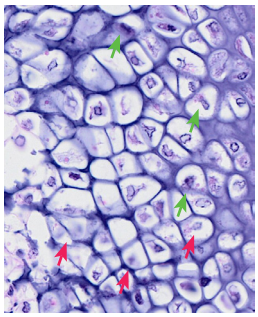
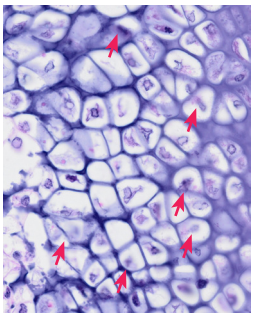
Incorrect



Focus surface for the entire image from one focus point in the center of the sample, leading to out-of-focus images.

Imaging system : Olympus VS200, iDS VS-264C color camera, 20x/0.8 NA objective lens

Sample: H&E-stained mouse pup section

Depth of Field (DF)		
Correct	**Acceptable Depending on Application**	**Incorrect**
		
40x/NA 0.95, DF 0.7 µm, extended depth of focus image (EDF/EFI) projection of a z-stack, 5 µm tissue When the DF is much smaller than the tissue thickness (3x or more) a z-stack is necessary to collect in-focus images from the entire tissue thickness and combining them in a projection image (EDF projection for color images and max intensity projection for IF images) so that all areas will appear in focus (compare areas indicated by green arrows between images).	20x/NA 0.8, DF 1.2 µm, one z-slice, 5 µm tissue section When the DF is 2-3x smaller than the tissue thickness, some areas can be out of focus (red arrows) when only one z-slice is imaged.	40x/NA 0.95, DF 0.7 µm one z-slice, 5 µm tissue section When the DF is much smaller than the tissue thickness (3x or more), extensive areas can be out of focus (red arrows) when only one z-slice is imaged.
Imaging system: Olympus VS200, iDS VS-264C color camera, 20x/0.8 NA and 40x/0.95 NA objective lenses Sample: H&E stained 5 µm thick mouse pup tissue section, zoom in on chondrocytes cells in the growth plate that will become spinal cord vertebrae		

References

- Visium HD FFPE Tissue Preparation Handbook (CG000684)
- Visium HD FF Tissue Preparation Handbook (CG000763)
- Visium HD FxF Tissue Preparation Handbook (CG000764)

Document Revision Summary

Document Number	CG000688
Title	Visium HD Spatial Applications Imaging Guidelines
Revision	Rev B
Revision Date	February 2025

Description of Changes

- Added additional considerations for the Thermo Fisher Scientific EVOS M7000 (p. 5)
- Added Troubleshooting section containing information on image compression and downsizing, image stiching, and focus issues (p. 10)
- Added references to Visium HD FF and FxF Handbooks (p.13)

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