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StrataMap Spatial Transcriptome

Imaging Guide

ILLUMINA PROPRIETARY

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Notice

Table of Contents

1. Overview

The StrataMap Spatial Transcriptome assay requires a qualified third-party imaging system and images of the stained tissue on the slide to meet quality standards. This guide provides the following information for qualifying an imaging system:

- Imaging system specifications
- Imaging systems that have been tested with the StrataMap Spatial Transcriptome assay
- Instructions for performing the imaging assessment that is used to qualify an imaging system

> *Illumina recommends using a slide scanner instead of a microscope for imaging. Slide scanners simplify the whole-slide H&E imaging by automating image acquisition. Slide scanners also maintain the image quality needed for downstream analysis and can be selected to accommodate a range of throughput needs.*

The guide also provides the following information on imaging during the StrataMap Spatial Transcriptome assay protocol:

- General imaging recommendations
- Image quality standards and troubleshooting recommendations

> *For imaging instructions, refer to StrataMap Spatial Transcriptome User Guide (document # 200073552) on the Illumina support site.*

1.1 Warnings, Cautions, and Notes

Icons are used for warnings, cautions, and notes across this documentation and are defined in Table 1.

Table 1: Icons

Icon	Definition
	Warnings are used for actions that can result in harm to person or damage to the instrument.
	Cautions are used for actions that can lead to unexpected or detrimental results, or loss of data.
	Notes are used for supplemental information that is required for a step, including Illumina recommendations and references to related documentation.

1.2 Safety Considerations

- Before performing the imaging assessment procedure, review the following applicable safety documentation:
  - Safety Data Sheets (SDSs)
  - Laboratory safety procedures
  - Equipment manufacturer instructions
- Personnel performing the imaging assessment procedure must be trained on the applicable laboratory safety practices and the operation of the equipment used in the steps.
- If compressed gas is used to dry slides, ensure that the gas source is properly regulated and used in accordance with local compressed gas safety requirements.
- Cryostat operation involves hazardous sharp edges and cold surfaces. Follow all safety precautions provided by the cryostat manufacturer and your facility safety procedures.
- Adhere to the following warnings and cautions when using samples and the reagents with this protocol.

> ***This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Ventilation should be appropriate for handling of hazardous materials in reagents. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations.*** *For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.*

> *Handle all samples as if they are potentially infectious agents.*

> *Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink, or smoke in designated work areas. Wash hands thoroughly after handling specimens and assay reagents.*

> *Exercise caution when handling sharps, including cryostat blades, razor blades, and scalpels. Follow institutional sharps safety procedures and dispose of used blades in approved sharps containers.*

Cryostat operation involves hazardous sharp edges and cold surfaces. Follow all safety precautions provided by the cryostat manufacturer and your facility safety procedures.

Do not use any assay components beyond their stated expiration date on the assay box label. Do not interchange assay components from different assay lots. Assay lots are identified on the assay box label. Store the assay components at the specified temperature.

Failure to follow the procedures as outlined can result in erroneous results or significant reduction in library quality.

2. Consumables and Equipment

Table 2 provides information on the required user-supplied consumables for performing the imaging assessment.

Unless otherwise specified, using substitute consumables and equipment can impact performance.

Table 2: Consumables

Consumable	Supplier
Absolute ethanol (EtOH), molecular biology grade	Decon, catalog # 2716 Sigma, catalog # E7023 (or equivalent)
Alcoholic eosin Y*	Sigma, catalog # HT110132 Abcam, catalog # AB246824 Leica, catalog # 3801615 or 3801616 Epredia, catalog # 71204
Absolute methanol (MeOH), molecular biology grade	Sigma, catalog # 34860-4L-R (or equivalent)
Bluing reagent*	Epredia, catalog # 6769001 Sigma, catalog # S5134-6X100ML Agilent, catalog # CS70230-2 Leica, catalog # 3802901
Conical tubes, 50 ml	General lab supplier
Glycerol	General lab supplier
H&E Staining Kit with the following consumables*: • Eosin Y solution, 30 ml • Bluing reagent, 30 ml • Hematoxylin, 30 ml	Abcam, catalog # ab245880
Hematoxylin*	Electron Microscopy Sciences, catalog # 26043-05 Sigma, catalog # MHS1, MHS16, MHS32, MHS80, MHS128, or 51275 Agilent, catalog # S330930-2 Leica, catalog # 3801520 Abcam, catalog # ab220365
Isopropanol	Sigma, catalog # PX1835-6 (or equivalent)

Consumable	Supplier
Lint-free wipes	General lab supplier
Microcentrifuge tubes, 1.5 ml	General lab supplier
Nuclease-free water	General lab supplier
Razor blade (or scalpel)	General lab supplier
Slide mailers, LockMailer	Simport, catalog # M9504MA
Trough, disposable plastic	General lab supplier

* Use either the H&E Staining Kit or the individual catalog numbers for alcoholic eosin Y, bluing reagent, and hematoxylin.

Table 3 provides equipment used for the imaging assessment that can be ordered from Illumina.

Table 3: Equipment

Item	Supplier
Razor blade/scalpel	General lab supplier
StrataMap Spatial Imaging Assessment Slide	Illumina, catalog # 20157069

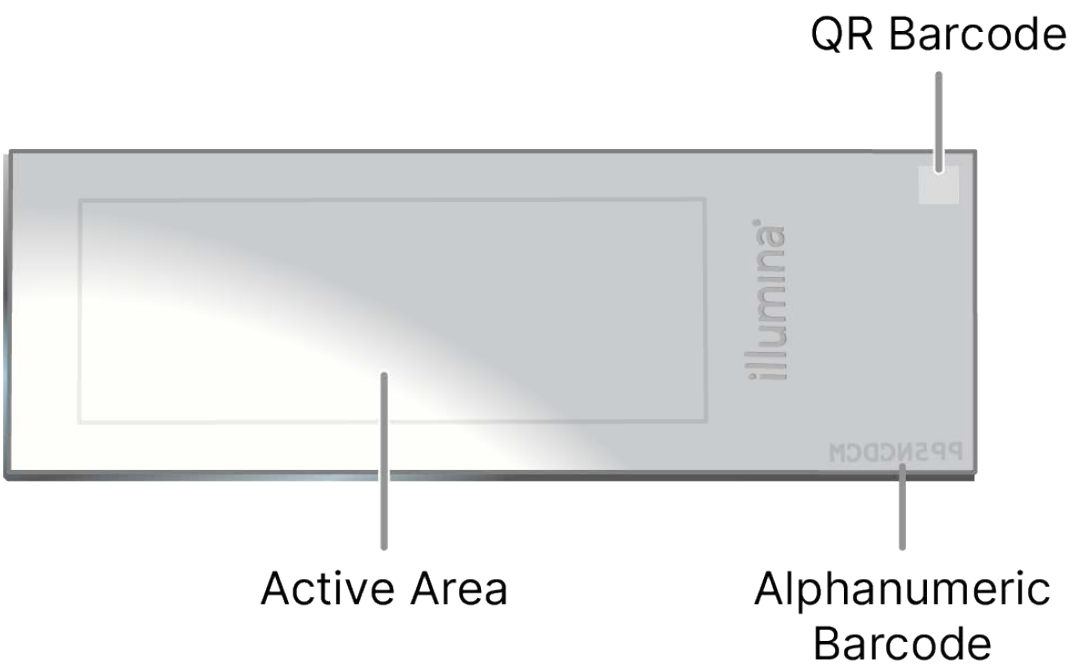
3. Tips and Techniques

This section identifies tips and techniques that can be used to perform actions before and during the imaging assessment.

3.1 Handle Tissue and Slide

- Identify the active side of the slide (Figure 1).
  - When the active side is facing up, the Illumina logo is legible.
  - The alphanumeric barcode is etched in the bottom-right corner of the slide. The barcode is not legible on the active side. Tissue sections are placed on this side of the slide.
  - If the barcode is legible, turn the slide over so that the active area is facing up.
  - If you are placing the slide on a surface, make sure that the active side is facing up.

Figure 1: Active Side of Slide



- When handling the slide, avoid touching the active area. Touch the edges of the slide only (Figure 2).

Figure 2: Slide Handling Example

- The slide is fragile. When handing the slide, do not apply excessive pressure.
- To avoid cross-sample contamination, make sure that tissue samples are placed at least 2 mm away from each other.
- If possible, place the tissue in the middle of the capture area and away from the edges.

3.2 Wash Slide

- The slide must be washed in nuclease-free water at specific points in the imaging assessment.
- Wash the slide with nuclease-free water as follows.

  1. Carefully place the slide into one of the prepared conical tubes with 45 ml nuclease-free water.

     When handling the slide, use gloves and touch the edges only. Do not touch the active area.

     For more information on how to handle the slide, refer to 3.1 Handle Tissue and Slide on page 7.

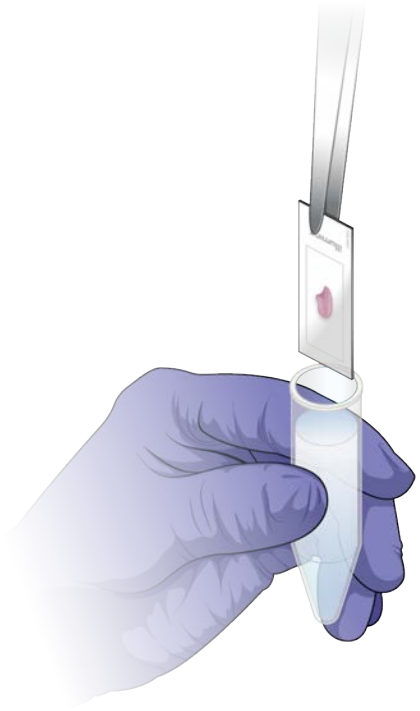
  2. Invert conical tube 15 times to wash.
  3. Using forceps, remove the slide from the conical tube.

- If you are having issues removing the slide, the following methods can help with removal:
  - Use the forceps to move the slide gently from side to side to loosen it from the tube wall.
  - Recap and invert the conical tube, and then gently tap the lid on the benchtop to release the slide from the bottom of the tube.

– Gently squeeze the sides of the conical tube to widen the area inside where the slide is stuck, and then use the forceps to remove the slide (Figure 3).

Make sure that the slide is not lodged in the cap before twisting to open the conical tube. Damage to the slide can occur.

Figure 3: Slide Removal



3.3 Apply and Remove Coverslip

- When placing a coverslip:
  - Use 75 µl 85% glycerol.
  - Pipette slowly to avoid bubbles.
  - If there are any bubbles on top of the tissue sections, gently press the coverslip onto the slide to remove them.

> *Do not shift the coverslip after mounting. Damage to the underlying tissue can occur.*

- Remove a coverslip as follows.

1. Place the slide in a prepared 50 ml conical tube with 45 ml nuclease-free water.
2. Incubate at room temperature for 5 minutes.
3. Using forceps, slowly remove the slide from the conical tube. As you lift the slide, make sure that the coverslip slides off.

4. If the coverslip does not slide off, submerge the slide in the nuclease-free water as much as possible and gently pull the coverslip parallel to the slide surface to separate it from the slide (Figure 4).

> *Do not pry or lift the coverslip at an angle or scrape the tissue sample.*

Figure 4: Coverslip Removal

3.4 General Imaging Recommendations

- For optimal image quality and spatial results, make sure that you are familiar with the imaging system and how it operates. Prior training with imaging systems and adhering to the imaging system specifications can help with acquiring optimal images.
- Illumina recommends using a slide scanner for imaging, as microscopes require manual adjustments, more experience with imaging principles, and additional training and practice for accurate results.

  Do not touch the imaging system while it is in operation. Do not move or shake the table that is holding the imaging system during imaging. Imaging errors can occur.

  For more information on stabilizing the imaging system during imaging, refer to the manufacturer instructions.

- Make sure that the slide is placed in the proper orientation for the imaging system.

  For more information on slide orientation and handling, refer to Tissue Sections and Handle Tissue and Slide in the StrataMap Spatial Transcriptome User Guide (document # 200073552) on the Illumina support site.

  - If the objective is in the top of the imaging system, place the slide active side up.
  - If the objective is in the bottom of the imaging system, place the slide active side down.

    If you are using a spring-loaded slide holder, release the spring slowly so that it does not crack the slide.

    For more information on the objective location, refer to the imaging system manufacturer instructions.

- Follow the imaging procedure from your imaging system manufacturer.

- The image must meet the following criteria:
  - Image is in focus.
  - Image is not overexposed or underexposed.
  - Image has good white balance and shading uniformity.

> *For more information on image quality and examples, refer to 3.5 Image Quality Standards on page 14.*

- Include one Field of View (FOV) border around the tissue edge.
- Stitch FOVs together using the software provided with your imaging system or a third-party application.
- Give each sample image a unique name. This name is used across all workflow steps. After the name is assigned, it cannot be changed.

3.5 Image Quality Standards

High-quality images are essential for accurate image alignment and downstream analysis.

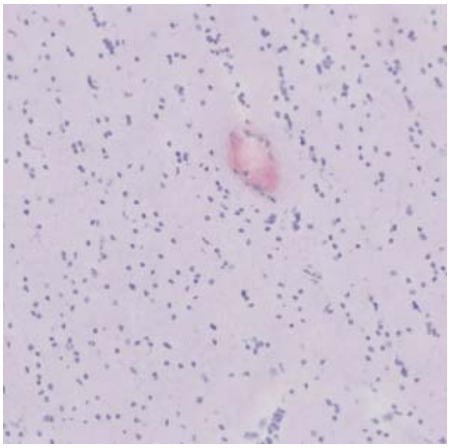
> *Illumina recommends assessing images visually before uploading them to the ISIT software.*
>
> *Some of the recommendations require washing the slide and applying a new coverslip. For instructions, refer to 3.3 Apply and Remove Coverslip on page 11 and 3.2 Wash Slide on page 9.*

Good Image Quality

Good image quality has the following attributes:

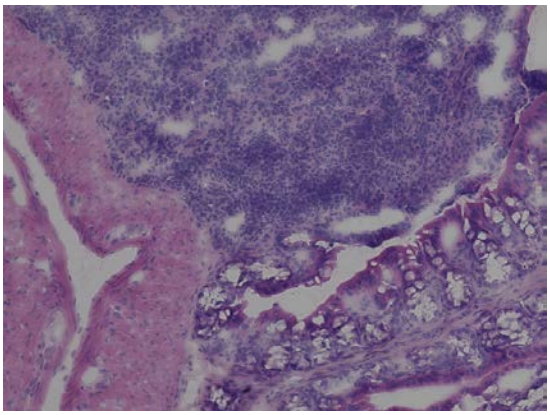
- Tissue sections have clearly defined nuclear borders (Figure 5).

Figure 5: Mouse Cerebellum with Defined Nuclear Borders



- Tissue sections have defined cellular and tissue structures (Figure 6).

Figure 6: Mouse Colon with Defined Tissue Structures



- Images have balanced exposure. Balanced exposure is sufficient brightness and contrast so that the visualization of the nuclei and tissue structures is clear and not overexposed.
- Tissue sections have consistent and precise thickness.

Focus Issues and Recommendations

Images should be in focus. If the image is significantly blurry, retake the image.

Images with slight blur are acceptable, but significant blurriness can impact cell segmentation analysis. Significant blur refers to the loss of cellular detail across the field of view (FOV).

Table 4 identifies blur issues and provides recommended corrective actions.

Table 4: Blur Issues

Issue	Potential Cause	Recommendation
Image appears blurry and blur is noticeable across the tissue (Figure 7 and Figure 8)	• Poor focus • Uneven tissue thickness across the imaging plane (Figure 10) • Debris on surface and/or coverslip	• Modify the existing focal points • Add additional focal points closer to the impacted region • If available, use auto-focus features • Make sure that the slide is flat when mounting onto the imaging system stage • If there is debris, remove the coverslip, wash the slide, and apply a new coverslip
Image has irregular or transparent blotches that obscure the image (Figure 9)	• Excess glycerol on the surface or coverslip • Glycerol pooling or bubbling • Objective lens is dirty or smudged (for example, with dust, oil, or glycerol buildup) • Moisture is present on the slide/sample	• For excess glycerol or glycerol pooling or bubbling, remove the coverslip, wash the slide, and apply a new coverslip • If the objective lens is dirty, inspect and clean the lens using the imaging system manufacturer instructions • If the tissue is not dehydrated, remove the coverslip, wash the slide, and apply a new coverslip

Example Images

The following example images show focus issues with human tissue. All images are at 20X magnification.

Figure 7: Slightly Blurred Human Tissue

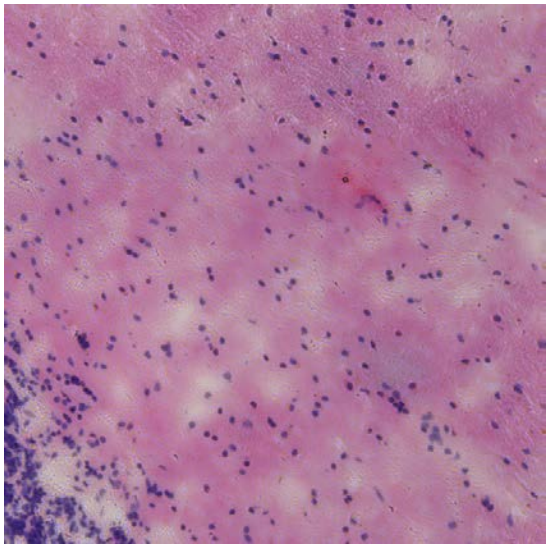


Figure 8: Out-of-Focus Human Tissue with Significant Blur

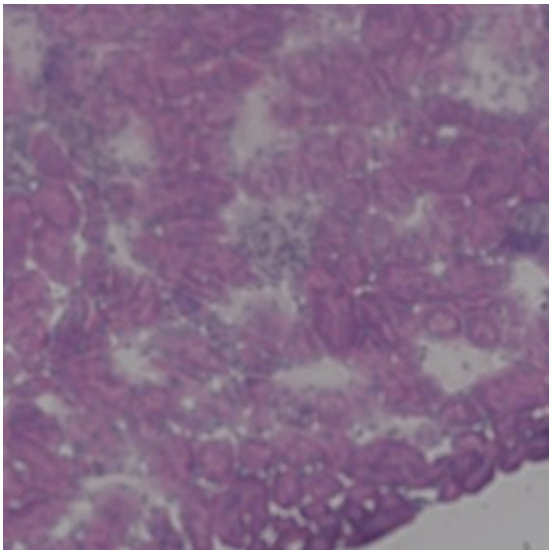


Figure 9: Human Tissue with Glycerol Bubbles

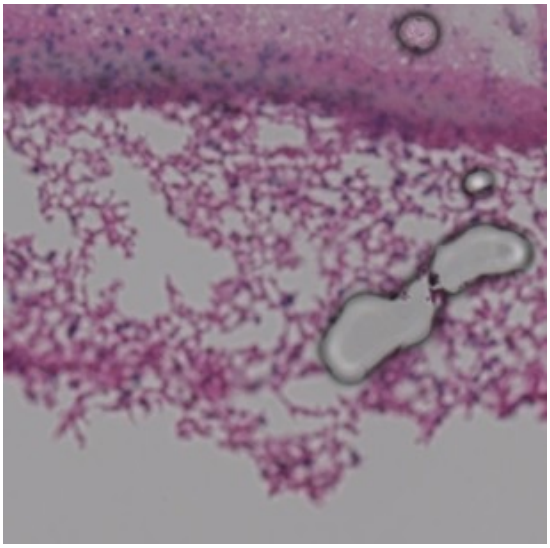
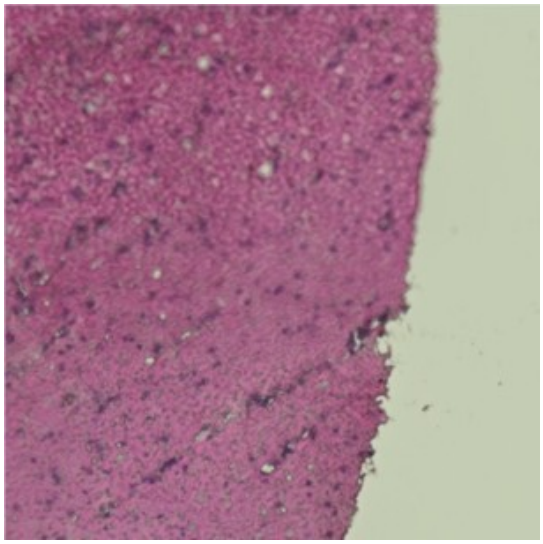


Figure 10: Blurred Human Tissue with Uneven Tissue Thickness



Exposure Issues and Recommendations

Exposure influences how much light is captured and balanced in the tissue and background. Improper exposure can negatively affect image quality.

Table 5 identifies exposure issues and provides recommended corrective actions.

Table 5: Exposure Issues

Issue	Potential Cause	Recommendation
Image is slightly dim (Figure 11)	Lower exposure setting or shorter exposure time	• Review the LookUp Table (LUT) to confirm that no areas are out of focus or blurry when the brightness and contrast settings are changed • If the nuclei and cells are visible, you do not need to increase the exposure setting or time

Issue	Potential Cause	Recommendation
Tissue is dark with poor contrast between cells (Figure 12)	Underexposure due to a low exposure setting or short exposure time	Use a higher exposure setting or increase the exposure time until the nuclei and cells are visible
Tissue is washed out or does not have enough detail (Figure 13)	Higher exposure setting or longer exposure time	Lower the exposure setting or shorten the exposure time
Fiducials are not visible through background brightness (Figure 13)	Higher exposure setting or longer exposure time	Lower the exposure setting or shorten the exposure time so that tissue detail and fiducial visibility are not impacted

Example Images

The following example images show exposure issues with mouse tissue. All images are at 20X magnification.

Figure 11: Slightly Dim Mouse Tissue

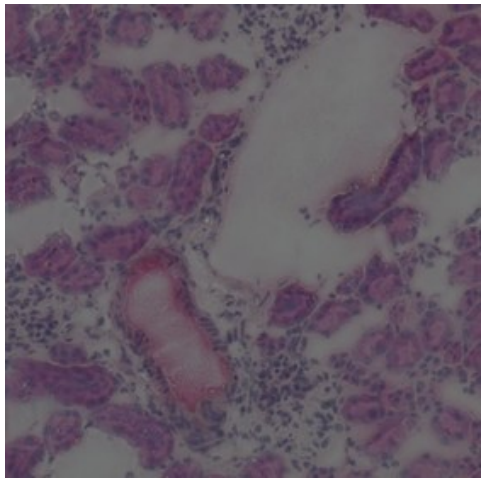


Figure 12: Underexposed Mouse Tissue

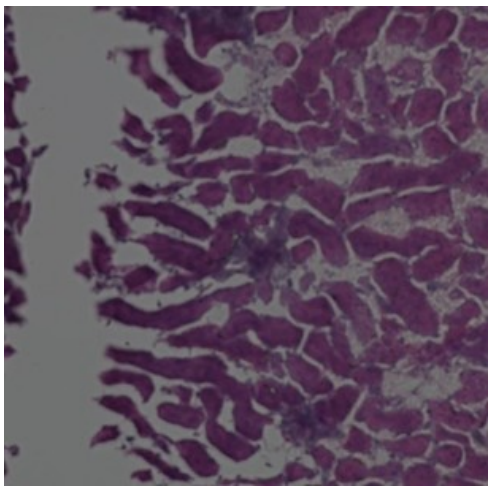
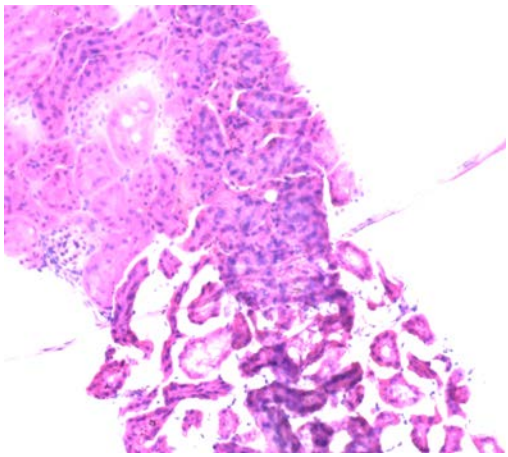


Figure 13: Overexposed Mouse Tissue



White Balance Issues and Recommendations

White balance can impact the hue of the image. If the tissue has an unnatural hue (for example, blue or yellow), then calibrate the white balance using the imaging system manufacturer instructions.

Shading Uniformity Issues and Recommendations

Shading uniformity makes sure that the entirety of the image is visible and evenly lit.

Table 6 identifies shading uniformity issues and provides recommended corrective actions.

Table 6: Shading Uniformity Issues

Issue	Potential Cause	Recommendation
Image has vignetting (bright center with dark edges)	Optical misalignment	Perform an optical realignment using the imaging system manufacturer instructions
Image has gradients or color shifting	• Optical misalignment • Dust or obstructions in optical components	• Remove additional filters, as needed • Perform an optical realignment using the imaging system manufacturer instructions • If the optical components have dust or obstructions, inspect and clean the components using imaging system manufacturer instructions

Stitching Issues and Recommendations

Stitching allows you to combine images to create a larger image.

Table 7 identifies stitching issues and provides recommended corrective actions.

Table 7: Stitching Issues

Issue	Potential Cause	Recommendation
There are visible seams or lines between image tiles	FOVs are not properly aligned during the stitching process	• Perform stitching with the stitching software for your imaging system or a third-party application • Do not do manual stitching • Make sure that there is sufficient overlap between FOVs. Illumina recommends a minimum of 10% overlap to support accurate alignment
There are gaps or missing image regions	Tiles were missing during image acquisition	• Make sure that the full imaging area is covered during acquisition • Review acquisition settings and confirm complete tile coverage
Fiducial or tissue identification fails in the stitched image	Stitching can introduce sharp edges or discontinuities that interfere with detection	• Perform stitching with software that minimizes edge artifacts • Inspect stitched images for abrupt transitions and adjust stitching parameters as needed
Images have misaligned or duplicate regions	Tile overlap percentage is set too low	Check and adjust the tile overlap setting. Illumina recommends a minimum of 10% overlap for proper alignment

Resolution Issues and Recommendations

Resolution affects image sharpness and how much detail is visible in an image. Lower resolution images can cause blur and other image issues.

Table 8 identifies resolution issues and provides recommended corrective actions.

Table 8: Resolution Issues

Issue	Potential Cause	Recommendation
The image quality is reduced after saving or exporting	• Acquisition software is compressing images, which causes resolution loss • Images are compressed during file conversion	• Save images with loss-less compression or as TIFF files to preserve the original resolution • Check for and turn off automatic compression in the export settings of the acquisition software
The software cannot handle the image resolution	• Resolution is too high • Pixel size is too small (for example, < 0.15 µm)	• Use 2x2 binning to reduce the resolution to a level that the software can handle
Images are blurry	• Resolution is too low • Image is out of focus	• Make sure that there is proper focus during imaging. If needed, use another imaging system with a higher optical resolution • Make sure that the pixel size is within acceptable limits
The cell or tissue features cannot be distinguished in the image	Resolution is too low or below the detection threshold	• Increase imaging resolution • Use an objective lens with higher magnification (for example, 20X)

Tissue Preparation Issues and Recommendations

Table 9 identifies tissue section and stain issues that can impact image quality and provides recommended corrective actions.

Table 9: Tissue Preparation Issues

Issue	Potential Cause	Recommendation
Image shows signs of stain bleeding in the slide (Figure 14)	• Slide has been stored for too long or stored improperly • Tissue is not sufficiently dehydrated	• Prepare and image tissue using a new slide • Make sure that slides are stored properly • If the tissue is not dehydrated, remove the coverslip, wash the slide, and apply a new coverslip
Tissue has dark red or purple blotches (Figure 15)	Blood lakes in the tissue section	• Flag affected sections in ISIT for downstream analysis • Exclude problematic tissue regions from analysis results
Tissue is pale or has uneven staining (Figure 16)	Tissue is not sufficiently dehydrated	If the tissue is not dehydrated, remove the coverslip, wash the slide, and apply a new coverslip
Tissue has visible folds or wrinkles in the image	Improper sectioning, mounting, or slide handling	• Flag affected sections in ISIT for downstream analysis • Exclude problematic tissue regions from analysis results

Example Images

The following example images show tissue preparation issues with human and mouse tissues that can impact imaging. All images are at 20X magnification.

Figure 14: Mouse Tissue with Stain Bleed

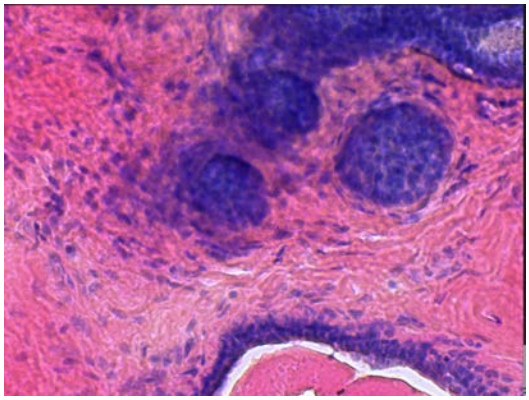


Figure 15: Human and Mouse Tissue with Blood Lakes

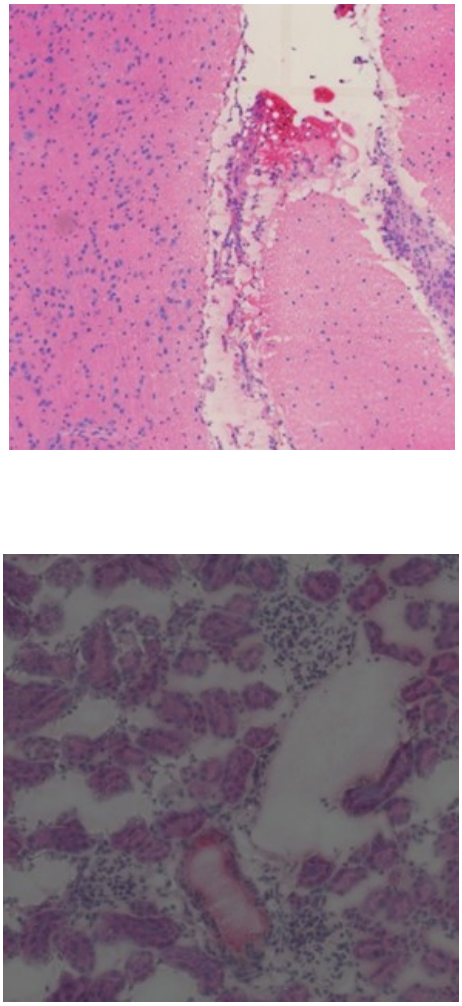
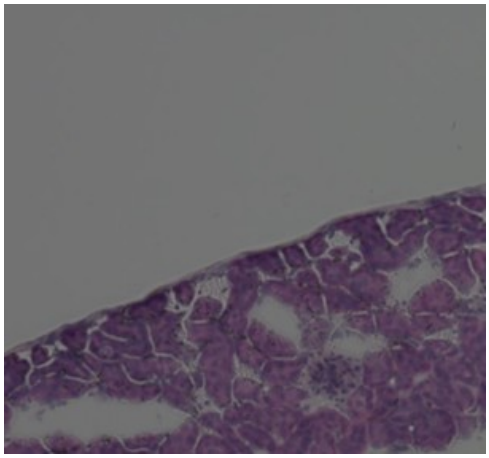


Figure 16: Insufficiently Dehydrated Mouse Tissue



4. Imaging System Specifications and Assessment

Before using an imaging system with the StrataMap Spatial Transcriptome assay, it must meet specifications identified in this section and pass the imaging assessment. This section also provides information on tested imaging systems that can be used with the assay or should be avoided.

4.1 Imaging System Specifications

Table 10 identifies specifications that are required for the imaging system used with the StrataMap Spatial Transcriptome assay.

> *If you are using a microscope, it must be qualified using the Microscope Assessment instructions in the Illumina StrataMap Spatial Software documentation.*

Table 10: Specifications

Item	Specification
Objective lens	Plan APO λ with 20X magnification and a numerical aperture (NA) between 0.4 and 0.8
Color camera	Capture color images for histology assessment (expected exposure time is 2–10 ms for good illumination)
White-balance functionality (RGB pixel)	Enabled
Sensor dimension	Greater than 1000 pixels on the shortest axis (X or Y)
20X FOV	Pixel size between 0.15 and 0.5 micron/pixel
Image output	Single-page, lossless (uncompressed), and in one of the following formats: • TIF/TIFF • OME-TIFF • QPTIFF • PNG • ND2 • SVS
Tile-scan overlap	Greater than or equal to 10%

Item	Specification
Scanning area	Covers an active area on the slide of 50 mm x 15 mm with the entire tissue sample is placed within the scanning area of the imaging system

4.2 Tested Imaging Systems

Table 11 lists example imaging systems that have been tested with the StrataMap Spatial Transcriptome assay. This list is not comprehensive and other imaging systems can be used if they meet the criteria outlined in *4.1 Imaging System Specifications* on page 30.

Table 11: Imaging System Examples

System	Type	Supplier
Aperio systems (AT2/CS2/CS5)	Slide scanner	Leica Biosystems
AxioScan 7	Slide scanner	Zeiss
BZ-X series (X710/800/810/1000)	Microscope	Keyence
DMi8	Microscope	Leica Biosystems
EasyScan One	Slide scanner	Motic
NanoZoomer 2.0 series (HT/S60)	Slide scanner	Hamamatsu
Ocus 20	Slide scanner	Grundium
PhenoImager (formerly Vectra Polaris)	Slide scanner	Quanterix (Akoya)
SLIDEVIEW systems (VS120/200)	Slide scanner	Evident (Olympus)

Table 12 lists imaging systems recommended by Illumina based on the amount of slides being processed.

Table 12: Imaging Systems, Recommended

System	Supplier	Additional Information
Aperio CS5 slide scanner	Leica Biosystems	Suitable for processing multiple slides at the same time.
Ocus 20 slide scanner	Grundium	Suitable for processing 1–2 slides at a time.

Table 13 lists tested imaging systems that are not recommended and should not be used with the StrataMap Spatial Transcriptome assay.

Table 13: Imaging Systems, Not Recommended

System	Supplier	Additional Information
Aperio GT series scanners	Leica Biosystems	Only compatible with fully cured coverslips and not wet slides.
EVOS M-series imaging systems (M3000/M5000/M7000)	Thermo Fisher Scientific	Observed image stitching errors and autofocus issues.

4.3 Imaging Assessment

The imaging assessment must be performed with the imaging system used with the StrataMap Spatial Transcriptome assay. This assessment also allows you to preview how the tissue type, staining, and sectioning performs with cell segmentation. The imaging assessment slide that is used has the same dimensions as a standard microscope slide and contains fiducial features that are used to evaluate key image quality metrics.

Images that are captured during the assessment are processed with the Illumina StrataMap Image Tool (ISIT) and analyzed with the Illumina StrataMap Cell Segmentation Tool in Illumina BioInsight Platform Core.

> *Use the same imaging system and objective lens that you plan to use for the StrataMap Spatial Transcriptome assay. If you are using a new imaging system or objective lens, or if capture settings change significantly, perform a new imaging assessment. Otherwise, issues with assay performance can occur.*

Consumables

- StrataMap Spatial Imaging Assessment Slide
- Absolute MeOH
- Alcoholic eosin Y
- Bluing reagent
- Glycerol
- Hematoxylin
- Isopropanol
- Nuclease-free water
- 1.5 ml microcentrifuge tubes
- 50 ml conical tube (qty 13–14)
- Cassette base
- Disposable trough
- Lint-free wipes
- No 1.5 coverslip
- **[Optional]** Pure compressed inert gas (for example, nitrogen gas)
- Razor blade (or scalpel)
- Slide mailer (qty 5)

Prerequisites

Before you begin the assessment, make sure that the following prerequisites are met:

- The pixel size (in µm) is recorded for the imaging system and acquisition settings being used. This information is required for accurate image analysis in ISIT.
- The imaging assessment slide is clean and not damaged.
- The imaging assessment slide is prepared with stained tissue sections that reflect your planned experimental setup.

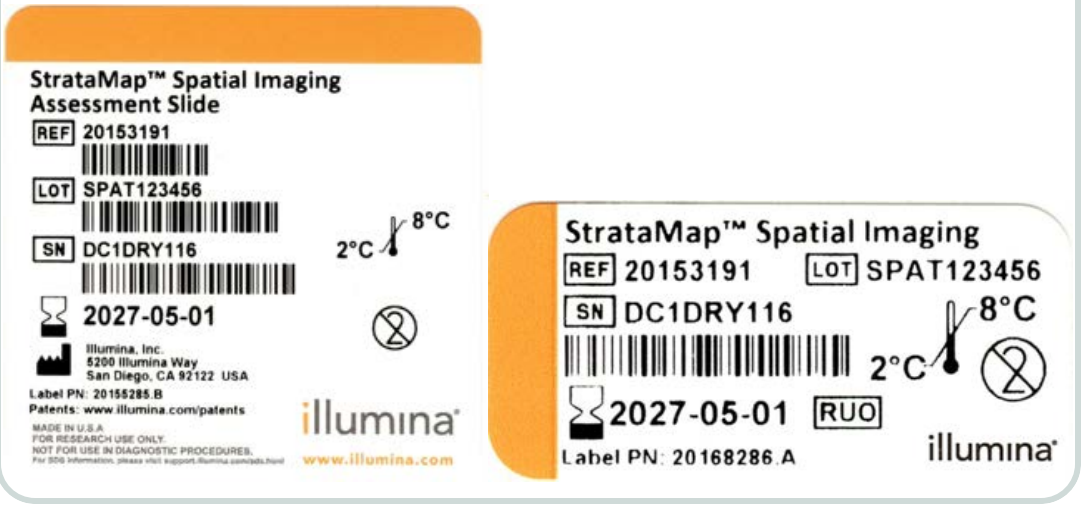
> *For optimal results, Illumina recommends using the same experimental conditions with the imaging assessment that are used with the StrataMap Spatial Transcriptome assay (for example, tissue identity and number of tissue sections).*

Preparation

1. Record the serial number of the imaging assessment slide packaging (Figure 17).

> *This serial number is used for image processing with ISIT and is identified as SN on the label. The same SN is used for the slide and the tube.*

Figure 17: Packaging Labels



Procedure

Section and Place Tissue

1. Turn on the cryostat to cool the chamber.

   For instructions, refer to the cryostat manufacturer manual.

2. Remove the tissue block from -80°C storage.
3. Place the tissue block into the cryostat for 30 minutes to equilibrate to the cryostat chamber temperature.
4. Prepare the MeOH as follows.
   a. Add 14 ml of absolute MeOH to a slide mailer.
   b. Place the slide mailer in the -20°C freezer for at least 30 minutes.
5. Remove the imaging assessment slide from the storage buffer.

   When handling the slide, make sure that you are using a sterile RNase-free technique to avoid contamination. Make sure that you are wearing gloves and only touch the edges of the slide to avoid the active area.

6. Gently wash the slide three times in nuclease-free water. Use a new conical tube for each wash.

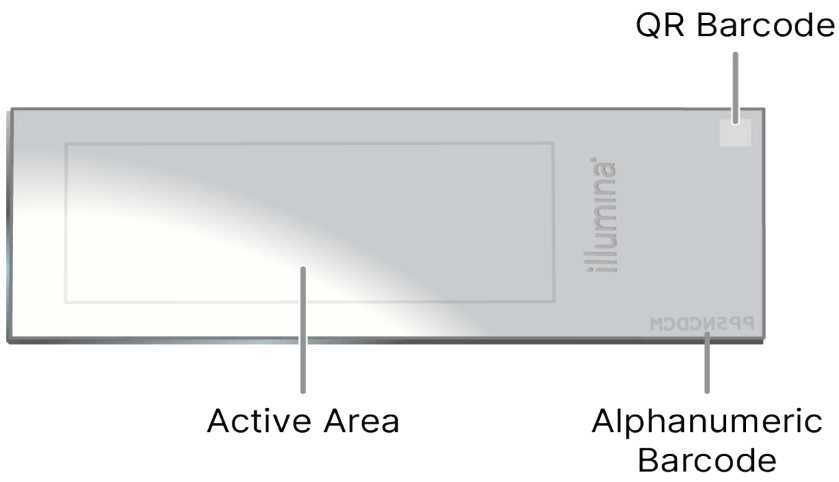
   For instructions, refer to 3.2 Wash Slide on page 9.

7. If you have pure compressed inert gas, use it to dry the slide.
8. If you do not have pure compressed inert gas, use a swinging bucket centrifuge to spin the slide in the conical tube at 250 x g for 30 seconds to remove excess water.

   When using the swinging bucket centrifuge, do not exceed 250 x g. Otherwise, the slide can break or become damaged.

9. Remove the slide from the conical tube and identify the active area.
   - When the active side is facing up and the slide is oriented horizontally, the QR barcode is in the upper-right corner.
   - The alphanumeric barcode is etched in the bottom-right corner of the slide. The barcode is not legible on the active side. Tissue sections are placed on this side of the slide.
   - If the barcode is legible, turn the slide over so that the active area is facing up.

Figure 18: Active Side of Slide



10. Place the slide active side up on top of a disposable trough and let it air dry until no water droplets remain.
11. Set the temperature of the cryostat blade and specimen holder.

Make sure that the cryostat reaches operating temperature before sectioning.

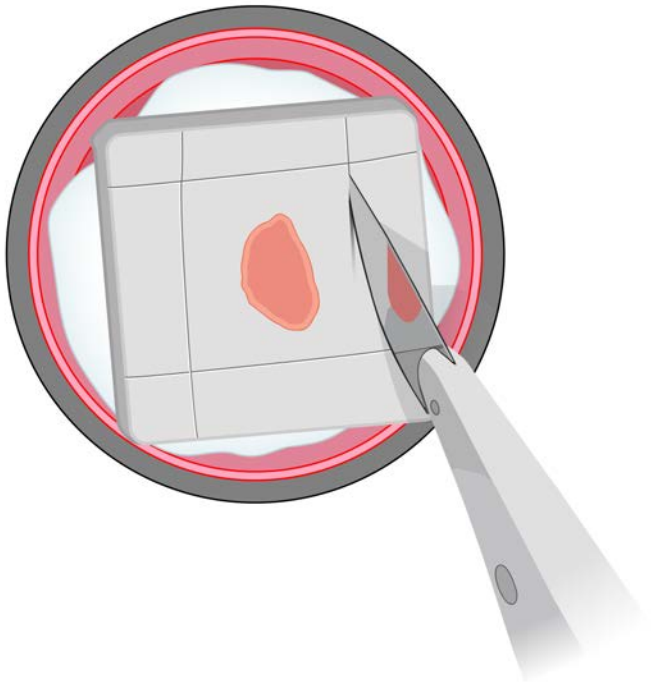
Illumina recommends -20°C for the blade holder and -10°C for the specimen head as starting points.

Adjust the temperatures as needed based on the tissue type or previous experience with using a cryostat for sectioning. For more information on adjusting temperature, refer to the cryostat manufacturer manual.

12. Place the specimen chuck with your tissue block into the specimen head of the cryostat.
13. Make sure that the OCT compound does not extend more than ~1 mm beyond the tissue edge. If it does, then use a razor blade or scalpel to carefully score so that excess OCT compound around the tissue can be removed (Figure 19).

> *Trimming excess OCT compound allows tissue to be positioned together closely, which maximizes the capture area.*

Figure 19: OCT Trimming



14. Install the cryostat blade.

> *To avoid injury when using the cryostat blade, make sure to follow all safety precautions and instructions in the cryostat manufacturer manual.*

> *Use a new blade for each tissue type.*

15. Slice tissue into 10 µm sections.

> *Make sure that the slide remains in the cryostat while working to avoid tissue thawing and degradation.*
>
> *To avoid decreased performance, make sure that the shortest amount of time possible (less than one hour) is used between mounting the first tissue section and fixing tissue.*

> *Illumina recommends discarding the first three sections cut from the tissue block so that highest quality tissue is available for mounting. For instructions, refer to the cryostat manufacturer manual.*

16. Place the tissue section on the slide surface as follows.

> *Make sure that you are placing the tissue section on the active side of the slide and avoid overlapping OCT with any neighboring tissue sections.*
>
> *Tissue sections cannot be adjusted or removed from the slide after placement.*

a. Pick up the slide and place a gloved finger on the back of the slide (the non-active side) where you are planning on placing the tissue section.
b. Hold your finger in place for at least 5 seconds to warm the slide before transferring the tissue section.

c. Lower the warmed active area of the slide onto the tissue section to start adhesion.

> *Do not touch the slide to the cryostat surface.*

> *The warmed area attracts the tissue section to the slide.*

d. Place a gloved finger on the back of the slide until the tissue section and OCT melts fully onto the slide.

e. Repeat steps a–d for the remaining tissue sections that must be added to the slide. Make sure that tissue sections are at least 2 mm apart.

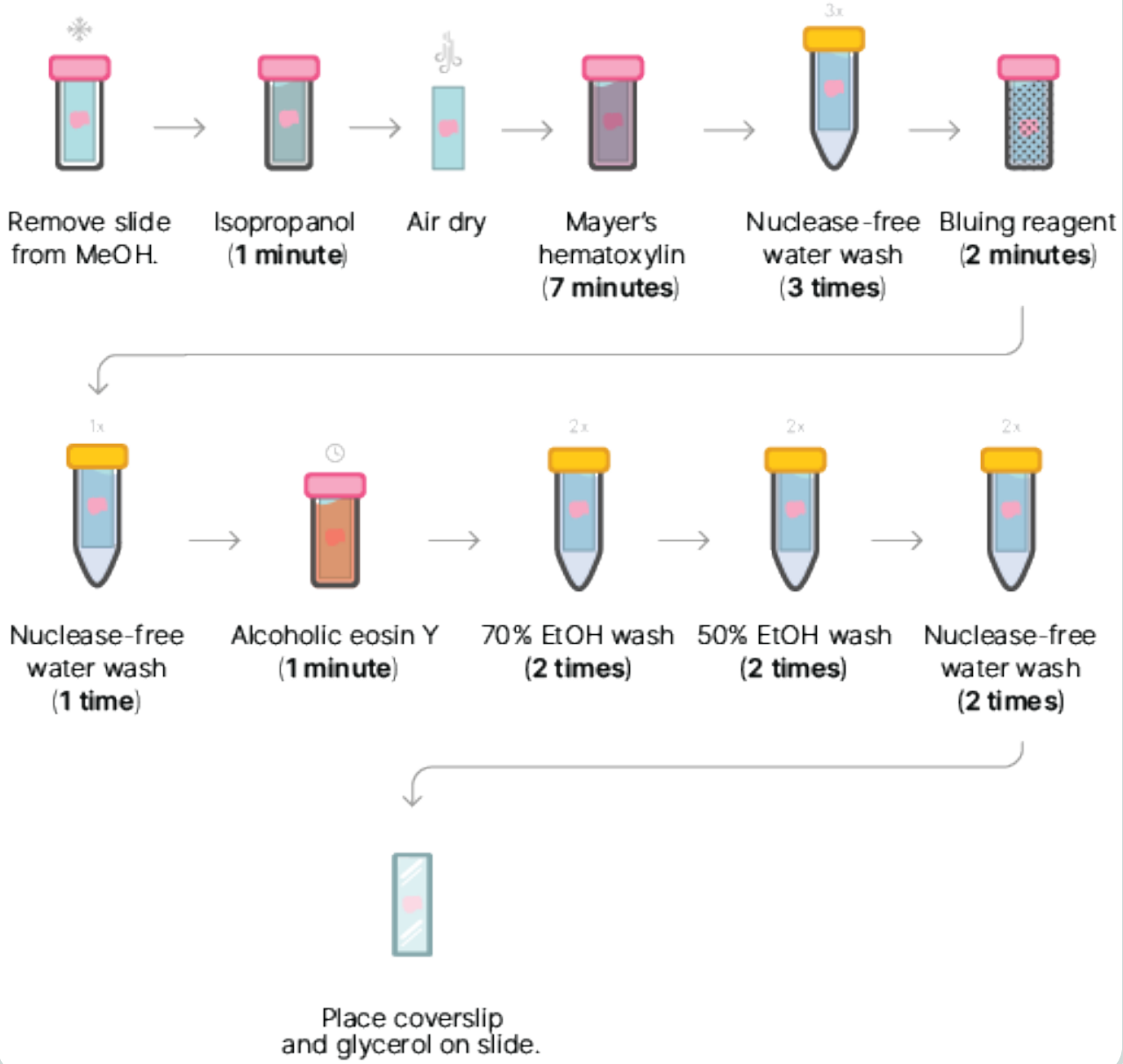
17. Immediately place the slide into the slide mailer with pre-chilled MeOH. Make sure that the tissue sections are fully submerged.

18. Place the slide mailer upright in the -20°C freezer for 30 minutes for tissue fixation.

Stain TIssue

This procedure involves incubating the slide in multiple reagents, as shown in Figure 20.

Figure 20: Stain Tissue Workflow



1. Remove the slide from the MeOH.
2. Prepare four slide mailers with reagents as follows.
   a. Add 14 ml isopropanol to a slide mailer.
   b. Add 14 ml hematoxylin to a slide mailer.
   c. Add 14 ml bluing reagent to a slide mailer.
   d. Add 14 ml alcoholic eosin Y to a slide mailer.
3. Add 45 ml nuclease-free water to six 50 ml conical tubes.
4. Combine the following volumes to create 100 ml fresh 70% EtOH, including overage. Vortex to mix.
   - Absolute EtOH (70 ml)
   - Nuclease-free water (30 ml)
5. Add 45 ml 70% EtOH to two conical tubes.
6. Combine the following volumes to create 100 ml fresh 50% EtOH, including overage. Vortex to mix.
   - Absolute EtOH (50 ml)
   - Nuclease-free water (50 ml)
7. Add 45 ml 50% EtOH to two conical tubes.
8. Combine the following volumes to create 300 µl 85% glycerol. Pipette slowly to avoid bubbles.
   - Absolute glycerol (255 µl)
   - Nuclease-free water (45 µl)
9. Preheat the thermal cycler to 37°C. Set the lid temperature to 47°C.
10. When prompted to select a reaction volume, set it to the maximum volume.

> *The volume is not applicable when using the cassette adapter.*

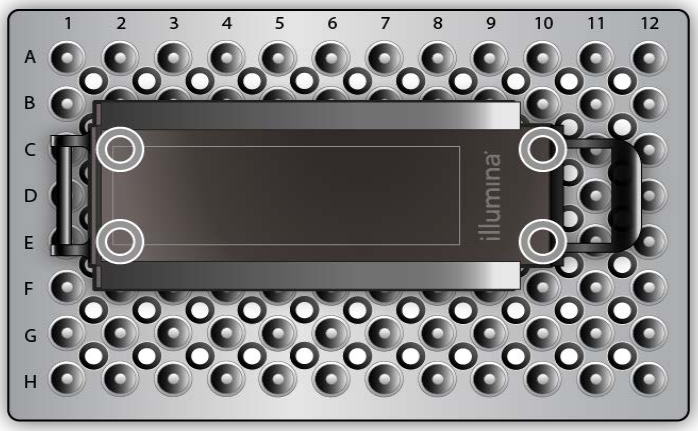
11. Remove the cassette from the packaging.

> *The cassette packaging includes the cassette base and lid. The cassette does not come pre-assembled.*

12. Place the cassette base onto the thermal cycler. Do not close the thermal cycler lid.

- The cassette base is used to dry the slide when it is placed in the thermal cycler.
- Make sure that the cassette is flat and that the cassette legs are within the wells of the thermal cycler (Figure 21).

Figure 21: Cassette Placement onto Thermal Cycler



13. Remove the slide from the MeOH. If the slide has excess MeOH on the surface after removal, you can proceed.

Do not touch the active surface. Damage to the sample or image quality issues can occur.

14. Incubate the slide in isopropanol as follows.

a. Carefully place the slide into the prepared slide mailer with 14 ml isopropanol.
b. Incubate at room temperature for 1 minute.
c. Using forceps, remove the slide from the slide mailer.

15. Place the slide active-side up on top of a trough and let air dry for 5–10 minutes. Inspect after 5 minutes.

> *Do not let the slide air dry for longer than 10 minutes. Damage to the sample or imaging quality issues can occur.*

> *The tissue appears white when it is dried.*

16. Incubate the slide in hematoxylin as follows.
    a. Carefully place the slide into the prepared slide mailer with 14 ml hematoxylin.
    b. Incubate at room temperature for 7 minutes.
    c. Using forceps, remove the slide from the slide mailer.

> *When using hematoxylin, avoid inhaling vapor or skin contact.*
>
> *For more information, refer to the SDS at support.illumina.com/sds.html.*

17. Wash the slide in nuclease-free water three times. Use a new conical tube for each wash.

> *For instructions, refer to 3.2 Wash Slide on page 9.*
>
> *If there is excess water on the surface after washing, you can proceed.*

18. Incubate the slide in bluing reagent as follows.
    a. Carefully place the slide into the prepared slide mailer with 14 ml bluing reagent.
    b. Incubate at room temperature for 2 minutes.
    c. Using forceps, remove the slide from the slide mailer.
19. Wash the slide in nuclease-free water one time.

> *For instructions, refer to 3.2 Wash Slide on page 9.*

20. Using forceps, remove the slide from the slide mailer.
21. Incubate the slide in alcoholic eosin Y as follows.
    a. Carefully place the slide into the prepared slide mailer with 14 ml alcoholic eosin Y.

b. Incubate at room temperature for 1 minute.

c. Using forceps, remove the slide from the slide mailer.

> *When using alcoholic eosin Y, avoid inhaling vapor or skin contact.*
>
> *For more information, refer to the SDS at support.illumina.com/sds.html.*

22. Wash the slide in 70% EtOH as follows.

a. Carefully place the slide into the prepared conical tube with 45 ml 70% EtOH.

b. Invert 15 times to wash.

c. Using forceps, remove the slide from the conical tube.

d. Repeat steps a–c one time for a total of two washes. Use a new conical tube for each wash.

23. Wash the slide in 50% EtOH as follows.

a. Carefully place the slide into the prepared conical tube with 45 ml 50% EtOH.

b. Invert 15 times to wash.

c. Using forceps, remove the slide from the conical tube.

d. Repeat a–c one time for a total of two washes. Use a new conical tube for each wash.

24. Wash the slide in nuclease-free water two times.

> *For instructions, refer to 3.2 Wash Slide on page 9.*

25. Place the imaging assessment slide in an empty conical tube.

26. Using a swinging bucket centrifuge, spin the conical tube containing the imaging assessment slide at 250 × g for 30 seconds to remove excess water.

> *Do not exceed 250 × g. Otherwise, the slide can break or become damaged.*

27. Remove the imaging assessment slide from the conical tube and let it air dry with the active side up for 5–15 minutes or until no water is present.

> *Monitor the imaging assessment slide closely during the drying process. If the slide does not fully dry, bubbles can form when applying the coverslip. Excessive drying can damage the tissue and affect image quality.*

28. Using glycerol, apply a coverslip to the imaging assessment slide.

> *Do not shift the coverslip after mounting. Damage to the underlying tissue can occur.*

> *For instructions, refer to 3.3 Apply and Remove Coverslip on page 11.*

29. **[Optional]** If a significant amount of bubbles form on the area covered by tissue, remove the coverslip, wash the slide two times in nuclease-free water, and apply a new coverslip.

> *For instructions, refer to 3.3 Apply and Remove Coverslip on page 11 and 3.2 Wash Slide on page 9.*

30. Incubate the slide at room temperature for at least 10 minutes to allow the glycerol to settle evenly.

31. Pick up the slide and lightly press it at an angle against a lint-free wipe to remove any excess glycerol that is seeping out from under the coverslip.

32. Using a lint-free wipe moistened with absolute EtOH, clean the back of the slide.

> *Excess glycerol must be removed before imaging. If glycerol is not removed, damage to the imaging system or image quality issues can occur.*

Imaging Assessment

1. Place the imaging assessment slide onto the imaging system stage. Make sure that the slide is properly aligned.
2. Start the imaging system software.
3. Make sure that 20X magnification is selected.
4. Capture an image of the entire tissue section on the imaging assessment slide. Make sure that the image encompasses at least 1 mm of white space around the tissue.

> *Make sure that the image is in focus and is not overexposed. Images that are overexposed or out of focus can cause poor fiducial detection results and image registration issues.*

For more information and best practices, refer to 4.1 Imaging System Specifications on page 30.

5. Save the brightfield color image as a single-page TIFF format or another media type that converts to TIFF (for example, BMP, ND2, CZI, LIF, or VSI) with an 8-bit or RGB format.

For compatible image formats, refer to 4.1 Imaging System Specifications on page 30.

6. Open ISIT and process the image.

For more information, refer to Image Processing in the Illumina StrataMap Spatial Software documentation.

7. Take a screenshot of the Image QC page.

The captured image and Image QC page screenshot can be shared with your Illumina representative.

8. Make sure that the sample files are exported to the Cloud so that the images are uploaded to Platform Core.

Use the standalone Illumina StrataMap Cell Segmentation Tool on Platform Core and the images processed with ISIT to perform cell segmentation and investigate the success of your sectioning, staining, and imaging. For more information on how to launch the pipeline and find key output files, refer to the Illumina StrataMap Spatial Software documentation.

5. Assay Imaging

After your imaging system has been assessed, it can be used for imaging with the StrataMap Spatial Transcriptome assay. After imaging, the Illumina StrataMap Image Tool (ISIT) is used to assess images.

For more information on ISIT, refer to the Illumina StrataMap Image Tool section of the Illumina StrataMap Spatial Software documentation.

After imaging, you can complete the remainder of the StrataMap Spatial Transcriptome assay protocol.

For more information, refer to the StrataMap Spatial Transcriptome User Guide (document # 200073552) on the Illumina support site.

Preparation

1. Make sure that the imaging system is set to 20X magnification.
2. Position the slide in the slide holder so that it is properly oriented for the imaging system.
   - For more information, refer to the imaging system manufacturer instructions.
   - If the objective is in the top of the imaging system, place the slide active side up.
   - If the objective is in the bottom of the imaging system, place the slide active side down.

   If you are using a spring-loaded slide holder, release the spring slowly so that it does not crack the slide.
3. Make sure that the imaging system is stabilized.

   Do not move or shake the table or touch the imaging system during imaging. Imaging errors can occur.

   For more information, refer to the manufacturer instructions.

Procedure

1. Image the slide using the imaging procedure from your imaging system manufacturer.
   - If the image contains more than one tissue section, crop the image so that there are individual images for each tissue section.
   - Include one Field of View (FOV) border around the tissue edge.
2. Using the software provided with your imaging system, stitch the FOVs together.

   You can also use a third-party application for FOV stitching.

3. Save the brightfield colored image as a single-page, uncompressed TIFF file with at least 8 bit depth.
4. Assign a unique name for the image (for example, slide ID_RepX).

   This name is used across all workflow steps. After the name is assigned, it cannot be changed.

5. Record the image pixel size in microns for use when processing the image with ISIT.
6. After imaging, assess the image visually and make sure that they have the following attributes:
   - In focus with no excessive blur
   - Clearly defined nuclear borders and cellular/tissue structures
   - Balanced exposure

   Good image quality is necessary for accurate image alignment and downstream analysis.

7. After visually inspecting the image, process the image using ISIT.

   tissue sections must be registered as separate samples in ISIT. For more information, refer to Image Processing in the Illumina StrataMap Spatial Software documentation.

8. Make sure that the image passes the Image QC step in ISIT, and then proceed to the remainder of the assay protocol.

> *Always visually inspect the image. An image passing the QC step in ISIT does not guarantee high-quality downstream analysis.*

9. **[Optional]** Use the Illumina StrataMap Cell Segmentation Tool to initiate cell segmentation before proceeding with the remainder of the assay protocol.

> *The Illumina StrataMap Cell Segmentation Tool can confirm if the cell segmentation meets your experimental needs or if the images should be retaken.*
>
> *For more information, refer to the Illumina StrataMap Spatial Software documentation.*

SAFE STOPPING POINT

- Slides can be stored in the following conditions:
  - Room temperature (~20–22°C) overnight (up to 16 hours)
  - 4°C for up to 48 hours
- Slides must be kept flat.
- Keep the coverslip and glycerol on the slide.

6. Resources and References

This section contains reference documentation and the revision history.

6.1 Reference Documentation

Table 14 identifies documentation that is used with the StrataMap Spatial Transcriptome assay or can be referred to for additional information on Illumina software.

Table 14: Reference Documentation

QR Code	Title
	StrataMap Spatial Transcriptome User Guide
	StrataMap Spatial Transcriptome Permeabilization Optimization Kit User Guide
	Illumina StrataMap Spatial Software documentation

6.2 Revision History

Document	Date	Description of Change
Document # 200081305 v01	August 2026	• Added references to StrataMap Spatial Transcriptome documentation. • Added Safety Considerations section. • Added recommended imaging systems. • Added assay imaging instructions.
Document # 200081305 v00	July 2026	Initial release.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website:	www.illumina.com
Email:	techsupport@illumina.com

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download from support.illumina.com.

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