User Guide | CG000549 | Rev D

Visium
CytAssist Spatial Training Kit

For use with:

Visium CytAssist Training Kit, PN-1000458

Take 1 minute to evaluate this protocol. Scan this code or click here.

Notices

Document Number

CG000549 | Rev D

Legal Notices

Instrument & Licensed Software Updates Warranties

Updates to existing Instruments and Licensed Software may be required to enable customers to use new or existing products.

Support

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Document Revision Summary

Document Number

CG000549

Title

Visium CytAssist Spatial Training Kit User Guide

Revision

Rev D

Revision Date

April 16, 2025

Description of Changes

- Updated tissue slide loading guidance in 1.3 Position Tissue Slides on the Tissue Slide Stage on page 18.

Table of Contents

Introduction

Objective

The purpose of this User Guide is to train new Visium CytAssist users on the following basic principles:

- Loading and aligning tissue slides onto the instrument.
- Loading a Visium Slide onto the instrument.
- Starting an experiment run on the instrument.
- Retrieving a Visium Slide from the instrument.
- Retrieving run data from the instrument.
- Cleaning the instrument.
- Assess alignment accuracy.

Some details, such as the preparation of the Visium slide, supported Capture Area size, supported tissue slide dimensions, etc, may differ from application to application. Consult the appropriate User Guide for detailed information.

This User Guide is used in conjunction with the Visium CytAssist Instrument Accessory Kit Quick Reference Card (CG000548)

For additional guidance, refer to the User Guides cited below:

- For guidance on qualifying the Visium CytAssist instrument, consult Visium CytAssist Specification Sheet (CG000570).
- For information on the Visium CytAssist Instrument, consult the Visium CytAssist Instrument User Guide (CG000542).
- For guidance on sample preparation or library construction, refer to the applicable Demonstrated Protocols and User Guides available on the 10x Genomics Support website.

Reagent Kits

Visium CytAssist Training Kit PN-1000458

Visium CytAssist Training Kit
PN-1000458
(store at ambient temperature)

	#	PN
Visium CytAssist Training Slide	2	2000681
Blank Slides	2	3000868

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Additional Kits, Reagents & Equipment

The items in the table below are validated by 10x Genomics and are highly recommended for the Visium Spatial Reagent Kits protocols. **Substituting materials may adversely affect system performance.** This list does not include standard laboratory equipment such as water baths, centrifuges, vortex mixers, pH meters, freezers etc. For some items, a number of options are listed. Choose item based on availability and preference.

For information on tested glass slides, refer to product-specific documentation.

Item	Description	Supplier	Part Number (US)
Plastics			
1.5 ml tubes	DNA LoBind Tubes, 1.5 ml	Eppendorf	022431021
	Low DNA Binding Tubes, 1.5 ml	Sarstedt	72.706.700
Pipette tips	Tips LTS 200UL Filter RT-L200FLR	Rainin	30389240
Wide Bore Pipette tips	Tips RT LTS 200UL FLW	Rainin	30389241
Kits & Reagents			
Eosin Optional, if Eosin staining	Refer to assay-specific documentation for recommended Eosins	-	-
Ultrapure Water	Ultrapure/Milli-Q water (from Milli-Q Integral Ultrapure Water System or equivalent)		
Equipment			
Pipettes	Pipet-Lite LTS Pipette L-100XLS+	Rainin	17014384
	Pipet-Lite LTS Pipette L-200XLS+	Rainin	17014391
Mini Centrifuge	VWR Mini Centrifuge (or any equivalent mini centrifuge)	VWR	76269-064
Chemical or Ethanol Resistant Marker or Pen *Optional, if drawing*	Fisherbrand Fine Tip Marking Pens	Fisher Scientific	13-379-4

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Tips & Best Practices

Icons

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

Pipette Calibration

- Follow manufacturer's calibration and maintenance schedules.
- Pipette accuracy is particularly important when using SPRIselect reagents.

CytAssist Training Slide Handling

- Wipe the back of the training slide with an ethanol-sprayed laboratory wipe. Do not use ethanol on the front of the training slide, as it may damage the spacers.
- Wipe the front of the training slide lightly with a laboratory wipe to remove excess liquid. Excess debris may be removed with compressed air, where permitted.
- Store training slide in a slide mailer at room temperature.

Visium Slide Handling

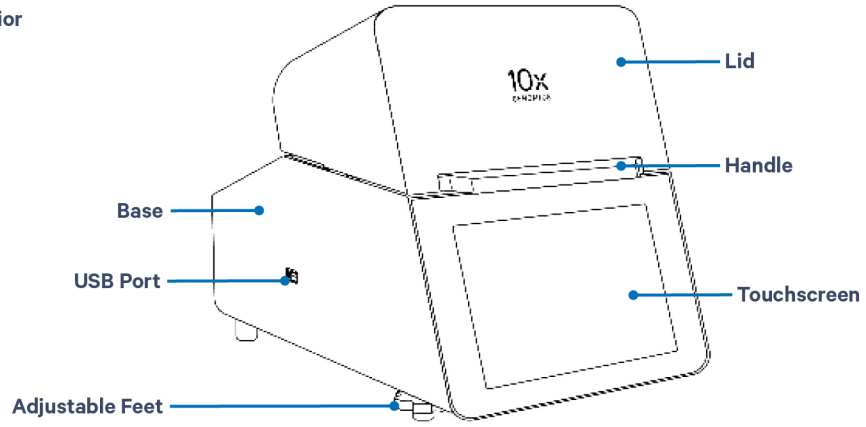
- Always wear gloves when handling slides.
- Ensure that the active surface of a slide faces up and is never touched. The active surface is defined by a readable label.
- Minimize exposure of the slides to sources of particles and fibers.
- When pipetting reagent onto a slide, avoid generating bubbles.

- Start the run immediately after the final reagent to the Probe Release Mix. While Probe Release Mix is not used in the Training Kit User Guide, minimize the time between Ultrapure water preparation and starting a run to mimic the sample run.

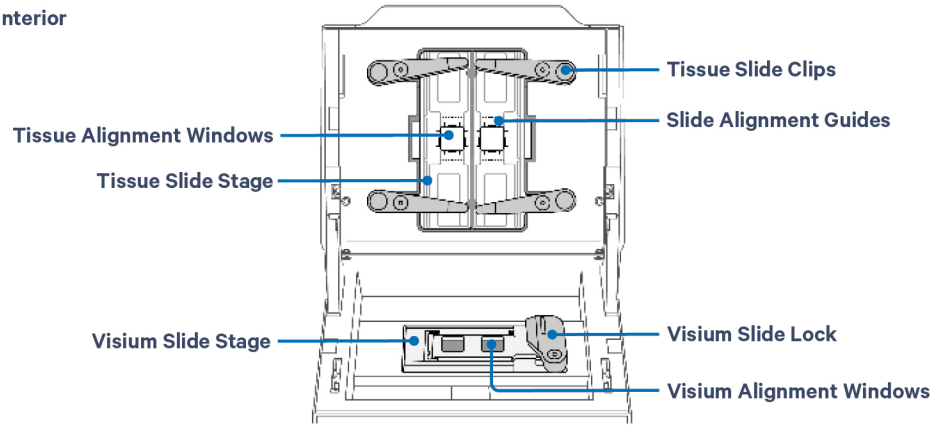
Visium CytAssist

Instrument Orientation

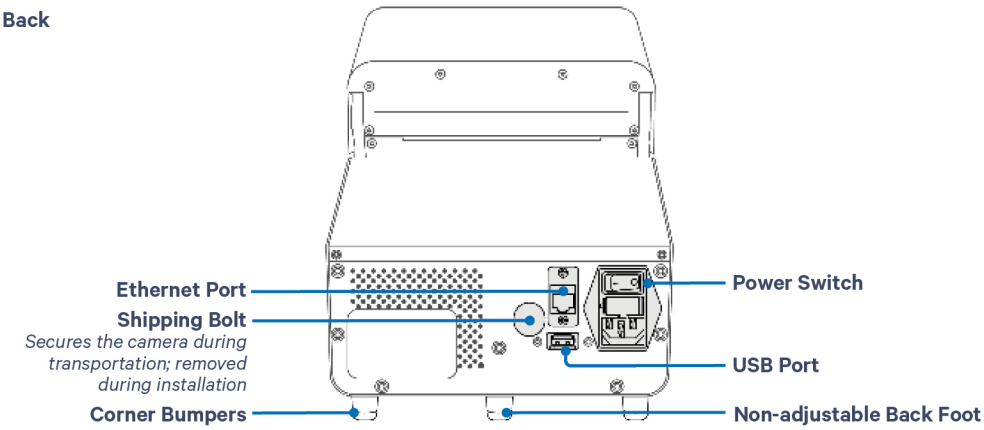
Exterior



Interior



Back



Step 1:

Training Step 1

1.0 Training Step 1

CHECKLIST – GET STARTED!				
Items		**10x PN**	**Preparation & Handling**	**Storage**
Obtain				
☐	Blank Slides (2) or Eosin Stained Tissue Sections on Glass Slides	-	-	Ambient
☐	Visium CytAssist Training Slides	2000681	-	Ambient
☐	Visium CytAssist Accessory Kit Quick Reference Cards (CG000548)	-	-	-
☐	Chemical or Ethanol Resistant Permanent Marker or Pen (only if drawing mock tissue)	-	-	Ambient

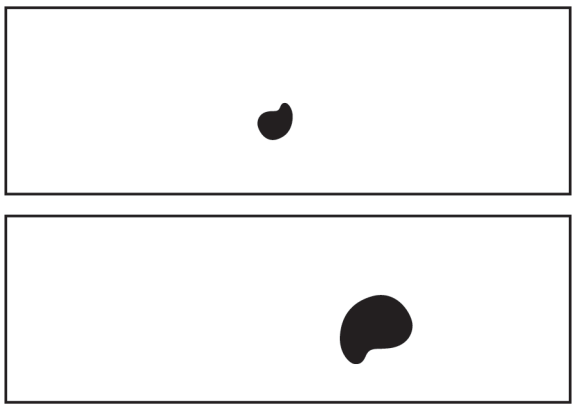
1.1 Draw Tissue

This step is unnecessary if performing the training workflow with eosin stained tissue sections on glass slides. The blank slides provided in the training kit are 25 mm wide. CytAssist training may be performed with any compatible slide. Refer to assay-specific documentation for a list of tested slides.

a. Overlay blank slides over the diagram on the Tissue Slide Alignment section of the Visium CytAssist Accessory Quick Reference Card (Document CG000548). If the Quick Reference Card has multiple slide options, choose the 25 mm Tissue Slide Alignment guidance. The tissue should lie within the green allowable area as shown in the quick reference card:

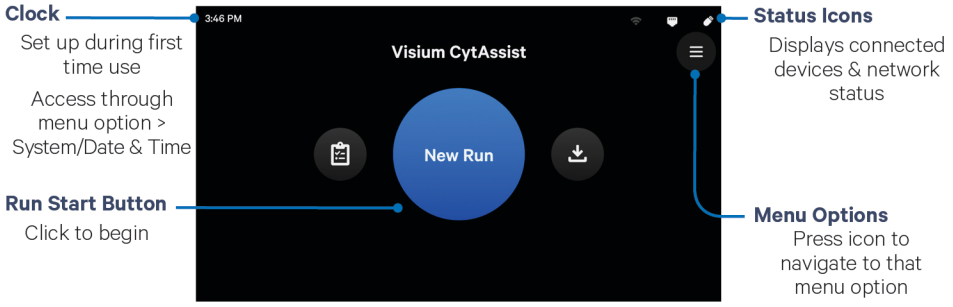
- 15 mm from top and bottom edges
- 5 mm from the sides

b. Using a permanent marker, draw a figure representing a tissue section within the green allowable area. The schematic below may be used to draw tissue that can fit within either the 6.5 mm or 11 mm Capture Area on the Visium CytAssist Spatial Gene Expression Slide.



1.2 Instrument Set Up

The home screen is the most common state of the instrument. There are several key functions accessible directly from the home screen.

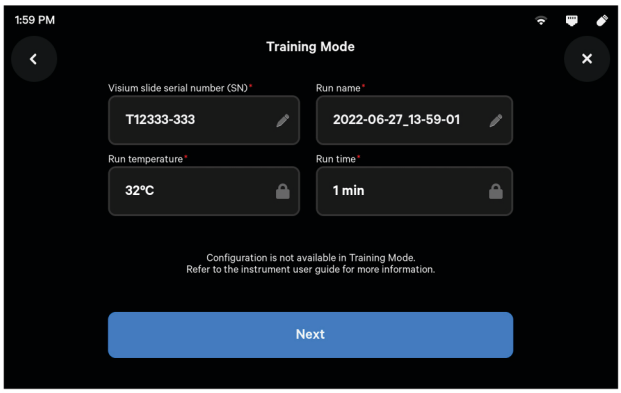


a. Press blue "New Run" Button on the touchscreen to initiate run.

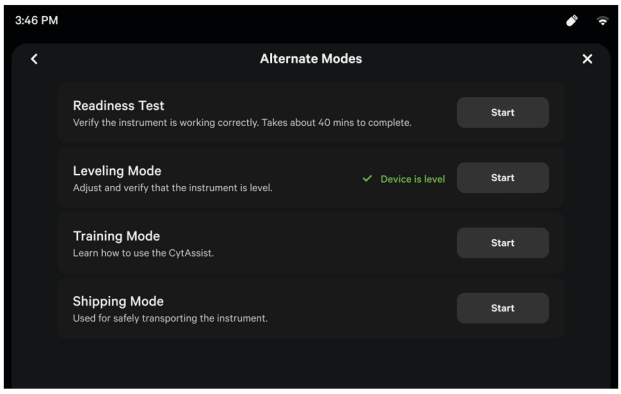
b. Enter new run information, including:

- Visium Slide serial number (entering a Visium CytAssist Training Slide serial number will automatically start Training Mode. Ensure serial number is accurate).
- Run name

Run temperature and run time are not editable during a training run.



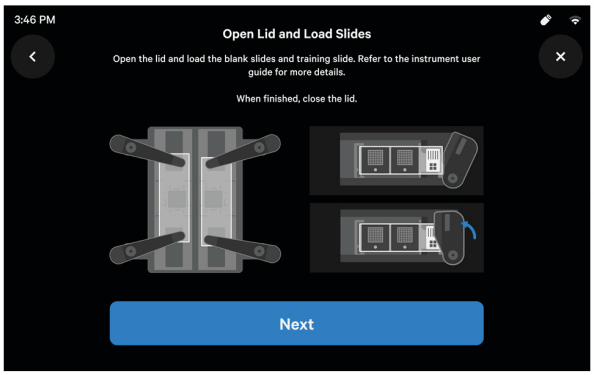
The Visium CytAssist may also enter training mode via the Alternative Modes menu. To access the Alternative Modes Menu, press the menu icon on the home screen, then press "System".



1.3 Position Tissue Slides on the Tissue Slide Stage

Tissue slide loading instructions are updated in this document revision (Rev D).

a. The CytAssist instrument should now display a Load Slides screen.

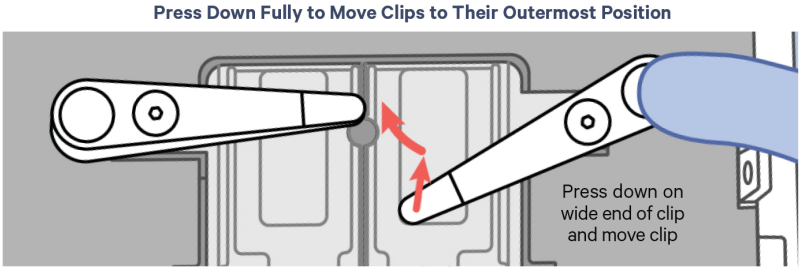


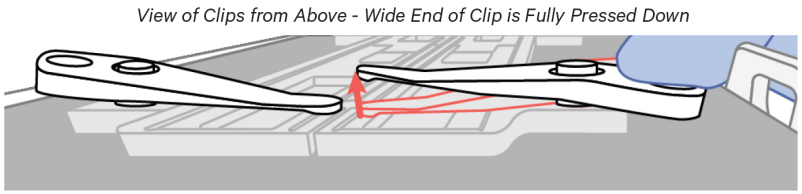
b. After entering the slide serial number, the instrument lid will unlock. Open the lid.

c. Wipe the back of the tissue slides with a laboratory wipe.

d. Press down fully on the wide end of each clip to lift and rotate the narrow end to its outermost position.

Cradle the instrument lid for increased stability and fully press down on the clips with the thumb. See image below for extent of clip rotation.

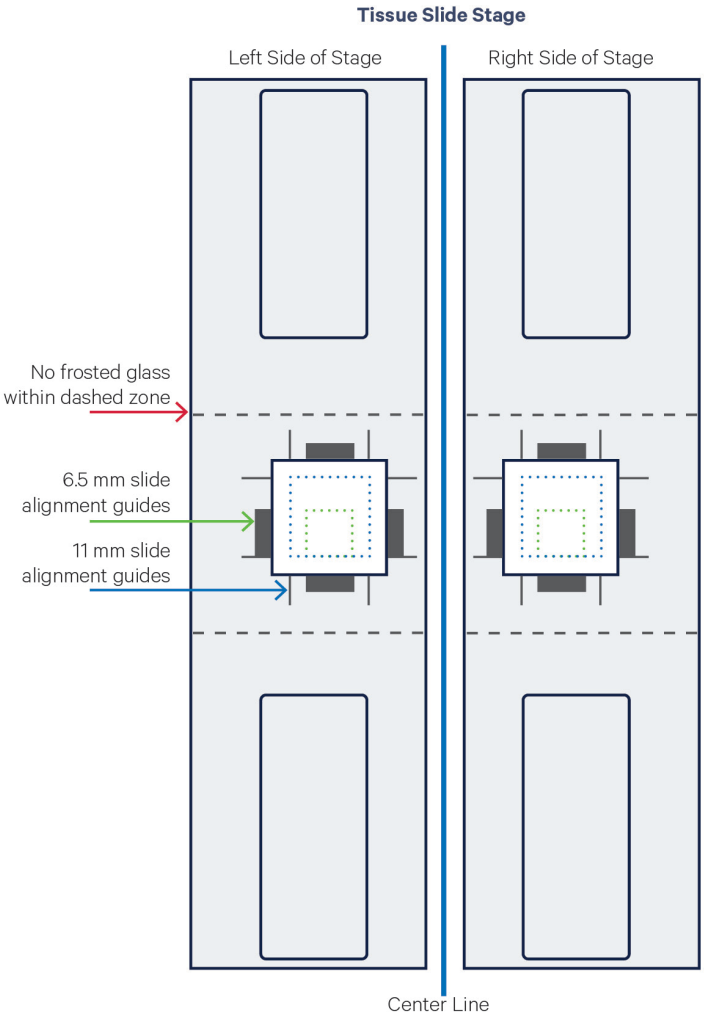




e. Lay the tissue slide flat on the stage surface and against the center line.

DO NOT touch the tissue section.

The image below may be used to verify if tissue or area of interest can fit within the appropriate alignment guides. If printing, select “actual size” or “100%” to print to scale. The blue Capture Area should measure 11 mm and the green capture area should measure 6.5 mm.



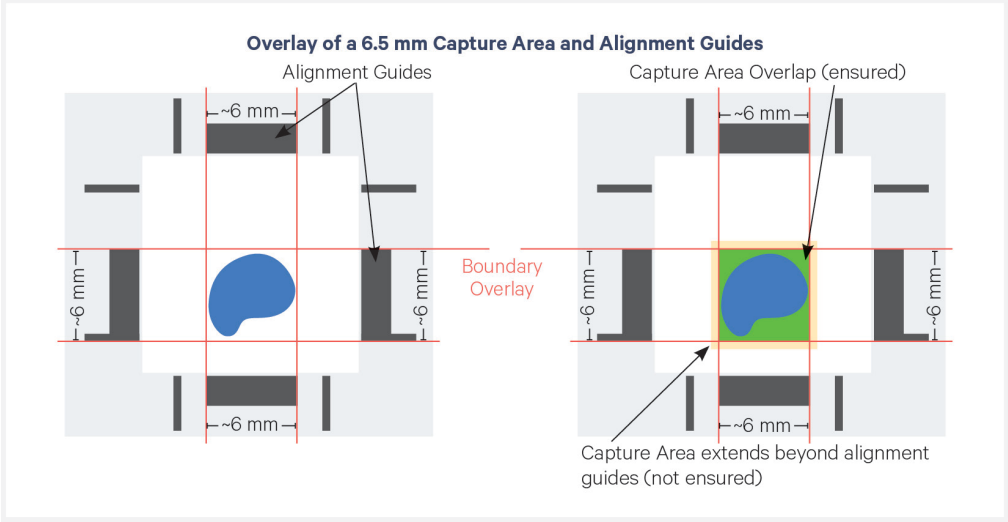
The dotted outlines of the Capture Areas that are shown in diagram are not present on the instrument’s surface.

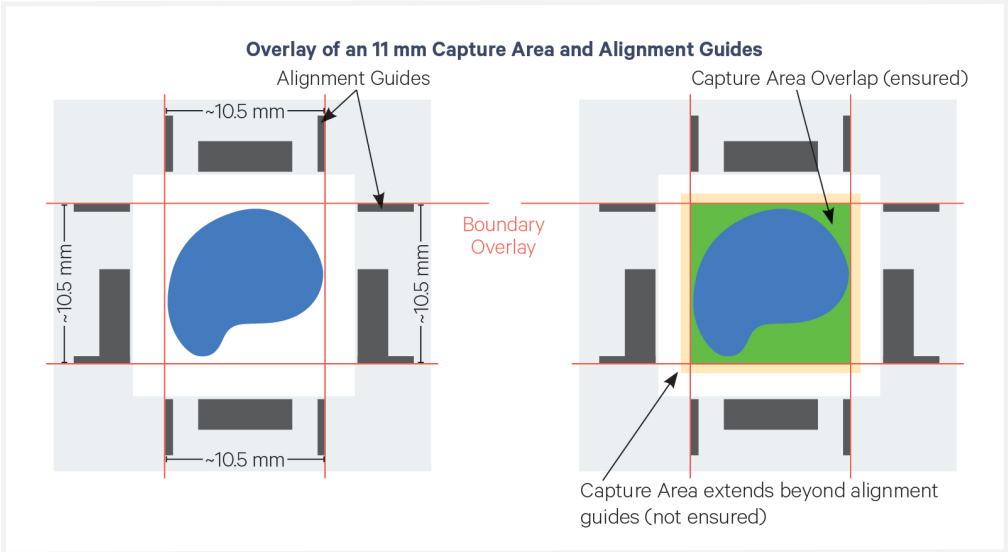
Slides should not overlap the center line.

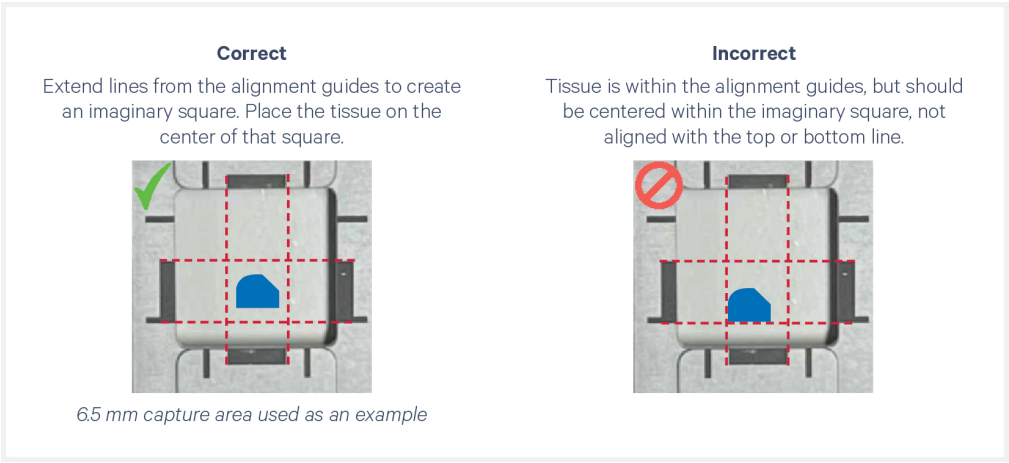
The **outside** of the alignment guides, shown in red, mark the inner boundaries of the fiducial frame on a Capture Area. They mark a distance **approximately** 6 mm (6.5 mm slides) or 10.5 mm (11 mm slides) wide on each side and surround the target region ensured to align within the fiducial frame.

When placing a tissue slide on the Tissue Slide Stage, center the tissue or area of interest within the area inside the outer edges of the alignment guides (area in green in the images).

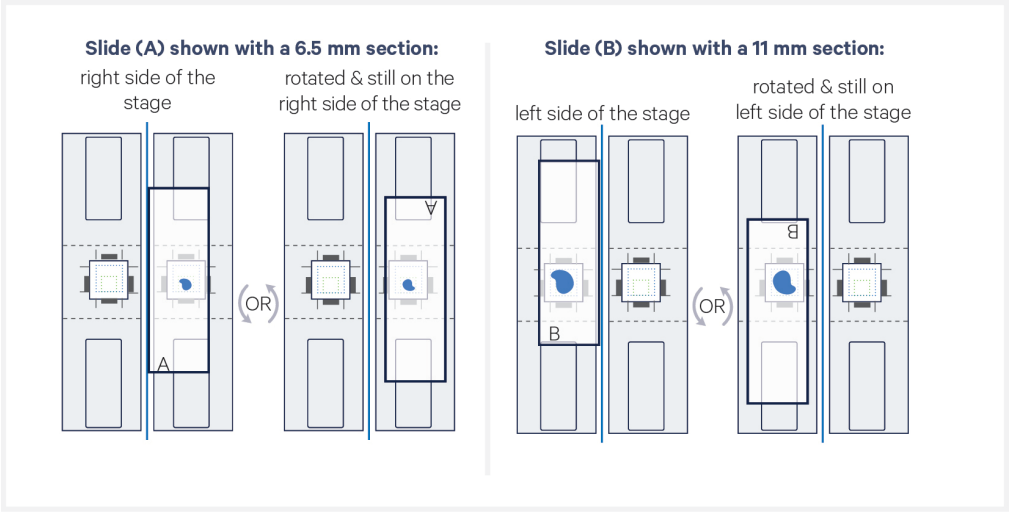
Alignment guide dimensions and locations are designed with a high tolerance to minimize data loss. Tissue within the alignment guides is ensured to overlap with the Capture Area during a successful run. Tissue outside of these guides may be captured (area in yellow in the images), but it is not ensured. 10x Genomics recommends placing high-priority tissue areas within the alignment guides.







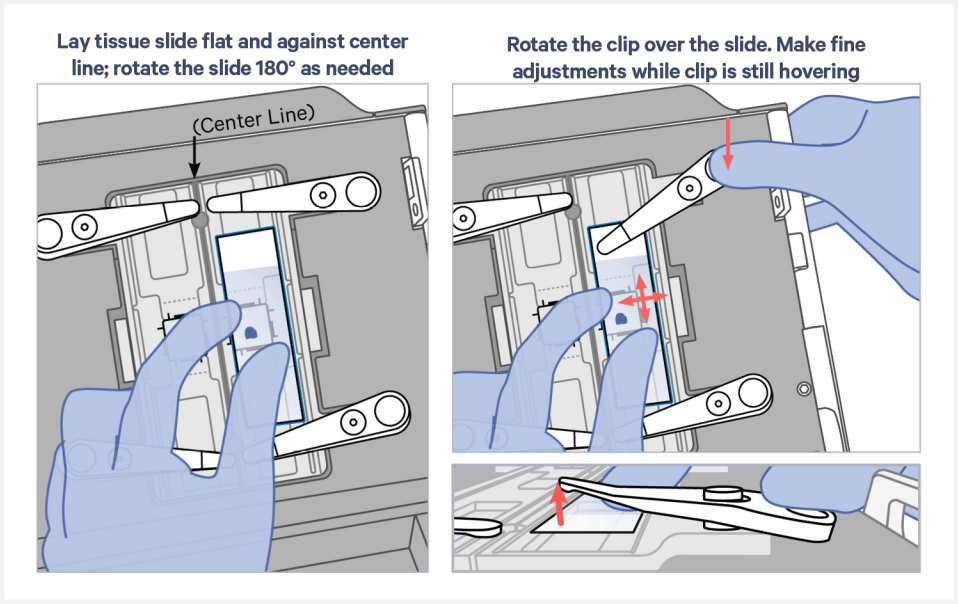
g. If necessary, rotate the slide 180° as shown to better place off-center tissues closer to the center line. Slides should not overlap the center line.



h. While clip is hovering over the slide, make fine adjustments to align the tissue or area of interest to the center of the alignment guides. Gently release when adjustments are complete.

DO NOT adjust slide while tissue clip is touching the slide, which may result in slide damage. DO NOT touch the tissue section.

DO NOT place clips on the edge of the slide, which may result in slide damage.



i. Move the first clip onto the slide and gently release when adjustments are complete.

   DO NOT place clips on the edge of the slide, which may result in slide damage.

j. Continue to hold the slide in place while pressing down fully on the wide end of the second clip. Rotate the second clip onto the slide and gently release.

   Use both clips whenever possible. The second clip may be omitted if the tissue slide is beyond its reach.

Step 2:

Training Step 2

2.0 Training Step 2

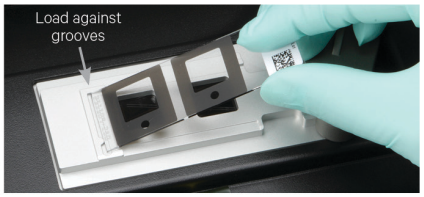
CHECKLIST – GET STARTED!				
Items		**10x PN**	**Preparation & Handling**	**Storage**
Obtain				
☐	Ultrapure/Milli-Q water (from Milli-Q Integral Ultrapure Water System or equivalent)	-	-	Ambient
☐	Blank Slides with Mock Tissue or Eosin Stained Tissue	-	Generated in Step 1	Ambient
☐	Visium CytAssist Training Slides	2000681	-	Ambient

2.1 Load Visium CytAssist Training Slide and Initiate Run

a. Load Visium CytAssist Training Slide within the grooves of the Visium Slide Stage and close Visium Slide Lock.

Open Visium Slide Lock

Load Visium Slide



Secure Slide with One Hand and Slowly Close Lock with the Other

Visium Slide Stage is Loaded Correctly with One Slide

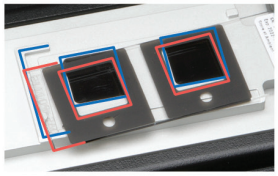
Slide fits inside the grooves.

Label on the right is partially obscured by the lock.

Capture areas line up with alignment windows.

INCORRECT

Slide does not fit inside the grooves. Capture areas do not line up with alignment windows.



INCORRECT

Label is on the left. Capture areas do not line up with alignment windows.

b. Add **75 µl** Ultrapure water to 1.5 ml microcentrifuge tube.

c. Centrifuge tube for 5 sec. Centrifugation is meant to mimic steps during a CytAssist assay sample run.

d. Dispense **25 µl** of water into each spacer well on the Visium CytAssist Training Slide. Do not depress after the first pipette stop to avoid generating bubbles.

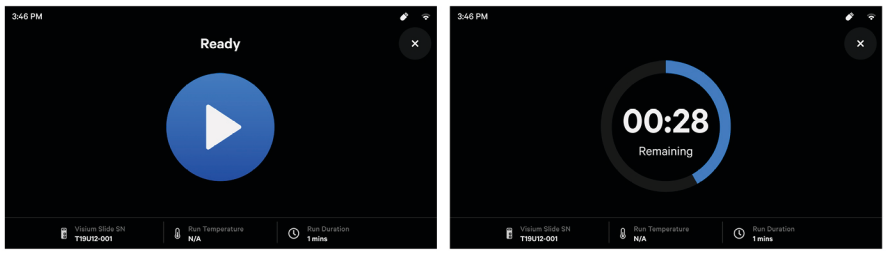
The time between reagent preparation and run initiation should be less than **5 min**. After adding reagent to the slide spacers, start run immediately.

e. Gently close the lid and press "Next".

The home screen will now display a play symbol and run information along the bottom of the screen.

f. Press the play button to start the run.

- Midrun progress bar will show the time remaining in the run.



g. At the end of a run, the button will display "Done" and a "Run Info" tab at the bottom of the screen.

h. Press "Done" and after the lid lock disengages, open the lid. DO NOT power off the instrument at this time, as it needs to process data.

i. Remove the Visium CytAssist Training Slide from the instrument. It is normal after a run for the slide to be wet.

The training slide may be reused.

During a non-training CytAssist run, users should IMMEDIATELY remove the Visium slide from the instrument and proceed with the protocol workflow.

j. Remove tissue slides.

k. Clean instrument. Refer to Visium CytAssist Instrument User Guide (CG000542) for instructions.

l. Proceed to Visium CytAssist Image Review.

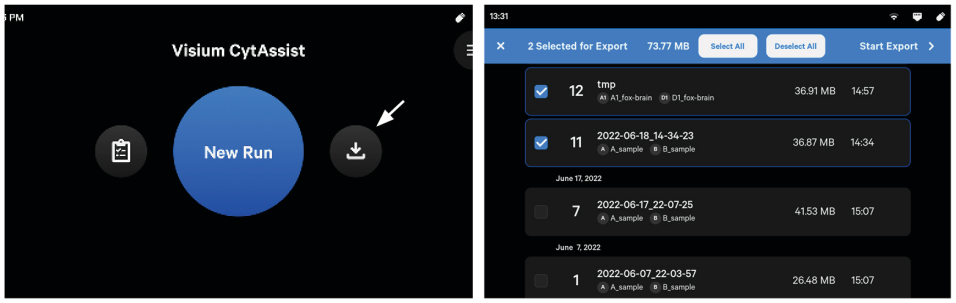
Step 3:

Training Step 3

3.0 Visium CytAssist Image Review

Run details and data export appear on the log page - data does not export automatically at the end of a run. During a typical Visium CytAssist experimental workflow, it is critical that users proceed directly to the next part of the assay workflow instead of initiating data export. Data export occurs in this User Guide directly after the instrument run for training purposes only.

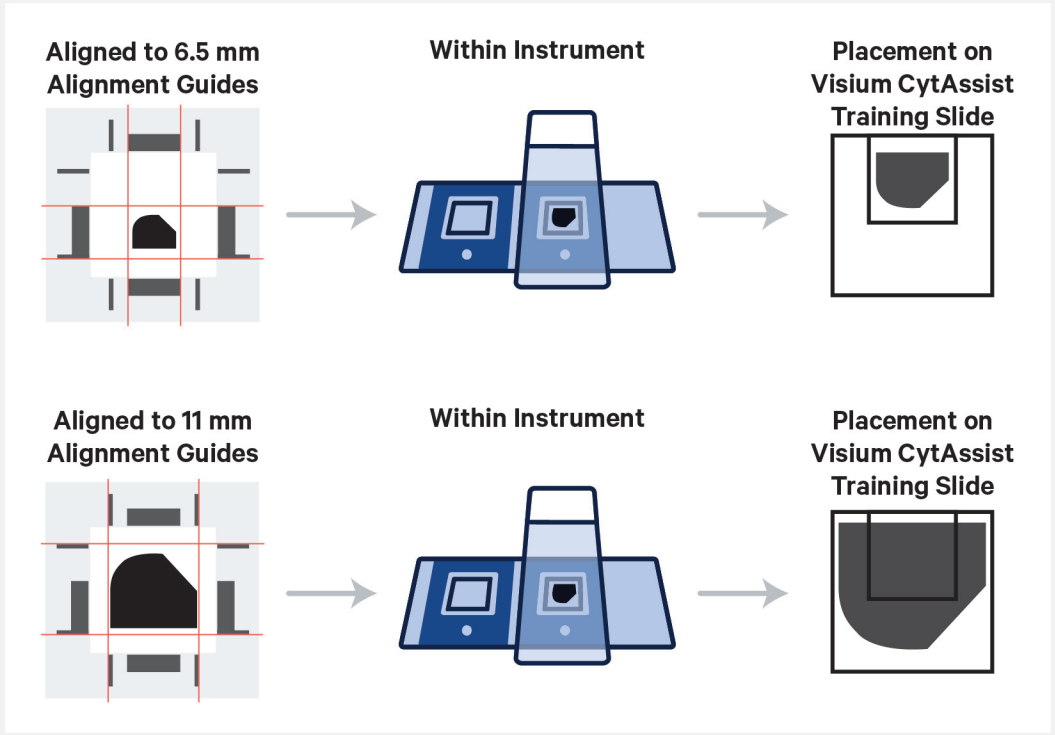
a. Once run is complete, export images via USB or Network Shared Folder using the export button on the home screen. Follow on-screen instructions. Data export will take approximately **2 min**. Refer to Visium CytAssist Instrument User Guide (CG000542) for more information.



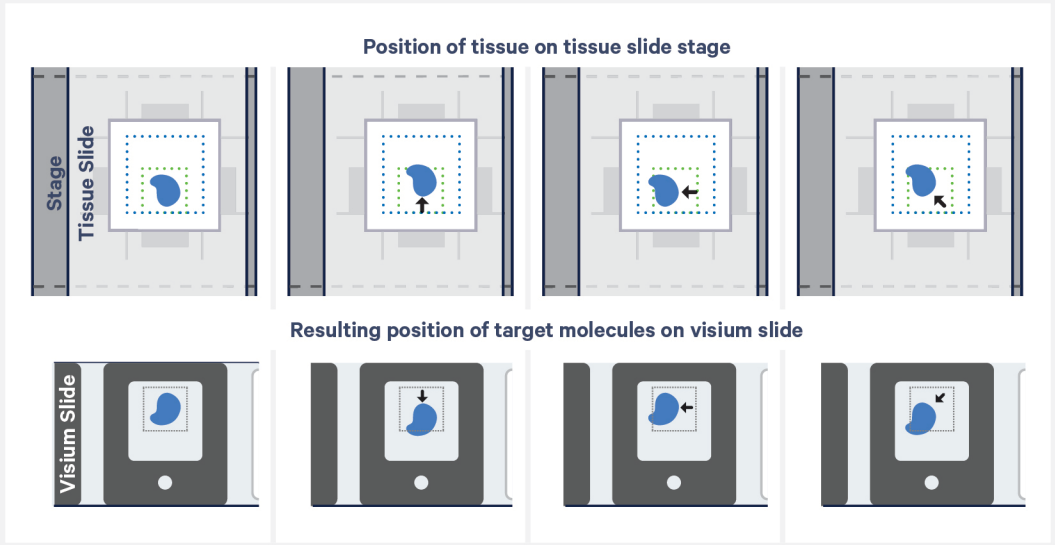
b. View exported images on a computer.

c. Assess accuracy of tissue alignment. If alignment was successful, the real or drawn tissue section should appear within the desired (6.5 mm or 11 mm) Visium training slide frame. Image should be free of artifacts such as bubbles. Images below are rotated.

The image below shows how tissues aligned to the alignment guides will translate onto the Visium CytAssist Training Slide when the instrument is closed.



The image below demonstrates how movement of the Tissue Slides affects where target molecules will end up on the Visium CytAssist Spatial Gene Expression slide. Use this information to correct misalignment of the tissue section.



Troubleshooting

Incorrect Visium Slide Loading

Loading the Visium Slide incorrectly onto the Visium Slide Stage may result in slide breakage. Ensure that the slide sits within the grooves of the Visium Slide Stage, with the label facing toward the right. The images below show the Visium Slide loaded correctly and incorrectly.

Visium Slide Stage is Loaded Correctly with One Slide

Slide fits inside the grooves.

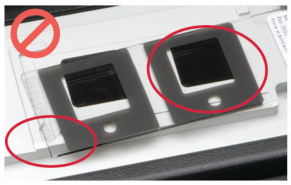
Label on the right is partially obscured by the lock.

Capture areas line up with alignment windows.

INCORRECT

Slide does not fit inside the grooves.

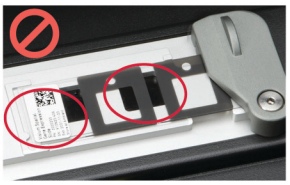
Capture areas do not line up with alignment windows.



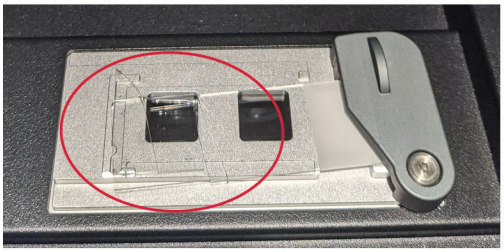
INCORRECT

Label is on the left.

Capture areas do not line up with alignment windows.

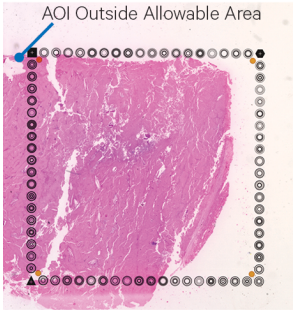
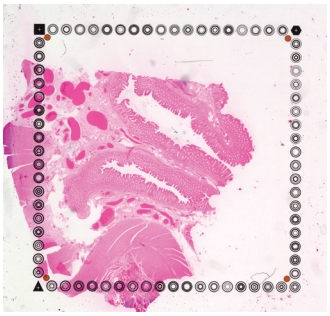


Slide is Broken due to Improper Loading



In the event of slide breakage, remove all traces of broken glass to avoid damaging the instrument. Exercise caution when removing glass to prevent injury.

Area of Interest Not Within Allowable Area

Area of Interest Outside of Allowable Area is Not Analyzed	
Tissue Larger than Capture Area	**Tissue Not Properly Aligned**
	

Areas of interest that are not placed within the allowable area on compatible glass slides will not be analyzed. This may occur if the tissue is larger than the Capture Area or if the tissue slide is not properly aligned when loading onto the Tissue Slide Stage. Tissue outside of the allowable area is fine if the tissue outside the allowable area is not an area of interest. See assay-specific documentation for information on tested glass slides.

Bubbles Trapped During Visium CytAssist Run

Avoid generating bubbles during reagent dispensing by pipetting slowly and avoiding expelling air from the pipette tip. If a bubble forms while dispensing reagent into the Visium CytAssist Training Slide, carefully pop the bubble with a pipette tip.