User Guide | CG000549 | Rev A

Visium
CytAssist Spatial Training Kit

For use with:

Visium CytAssist Training Kit, PN-1000458

Notices

Document Number

CG000549 | Rev A

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

Email: support@10xgenomics.com
10x Genomics
6230 Stoneridge Mall Road
Pleasanton, CA

Document Revision Summary

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Title
Visium CytAssist Spatial Training Kit User Guide

Revision
Rev A

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July 18, 2022

Specific Changes

General Changes

Table of Contents

Introduction

Objective

The purpose of this User Guide is to train new Visium CytAssist users on:

- Loading and aligning tissue slides onto the instrument.
- Loading Visium CytAssist Spatial Gene Expression Slides onto the instrument.
- Starting an experiment run on the instrument.
- Retrieving the Visium CytAssist Spatial Gene Expression Slide from the instrument.
- Cleaning the instrument.

At the end of the training workflow, users will assess tissue alignment accuracy.

This User Guide is used in conjunction with the Visium CytAssist Instrument Accessory Kit Quick Reference Cards (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 guidance on sample preparation for library construction and sequencing, refer to the applicable Demonstrated Protocols (CG000518, CG000519, CG000520) and User Guide (CG000495) available at the 10x Genomics Support website.

Reagent Kits

Visium Spatial Gene Expression for FFPE Reagent Kits

Refer to SDS for handling and disposal information.

Visium CytAssist Training Kit PN-1000458

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

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

Additional Kits, Reagents & Equipment

The items in the table below have been 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.

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
Kits & Reagents			
Eosin	Eosin Y solution, alcoholic	Millipore Sigma	HT110116
	Eosin Y Solution (Modified Alcoholic)	Abcam	ab246824
	Eosin Y with Phloxine 1% alcholic solution	VWR	10143
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	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

Indicates a version specific update in volume, temperature, instruction, etc.

Pipette Calibration

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

Visium Spatial Slide Handling

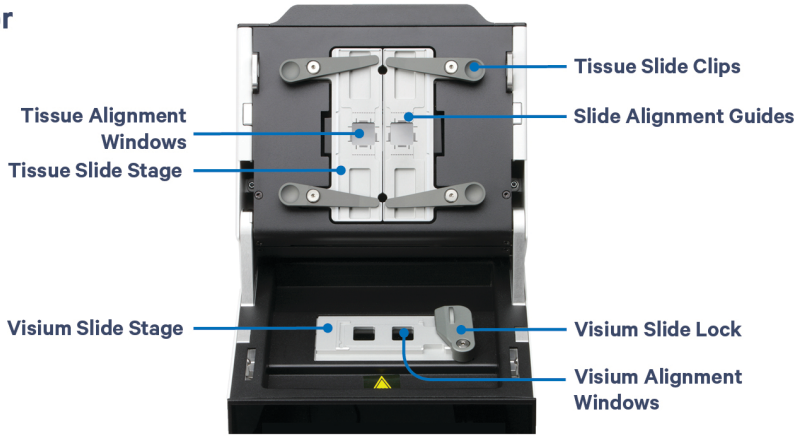
- Always wear gloves when handling slides.
- Ensure that the active surface of a slide faces up and is never touched. The active surface is defined by a readable label.
- Minimize exposure of the slides to sources of particles and fibers.
- When pipetting reagent onto a slide, avoid generating bubbles.
- Time between adding Probe Release Mix onto spacers on the Visium CytAssist Spatial Gene Expression v2 Slide on the Visium CytAssist instrument and starting a run should not exceed **5 min**. While Probe Release Mix is not used in the Training Kit User Guide, the time between reagent addition and starting a run should also be kept to under **5 min** to mimic the assay.

Visium CytAssist

Instrument Orientation

Exterior

Interior



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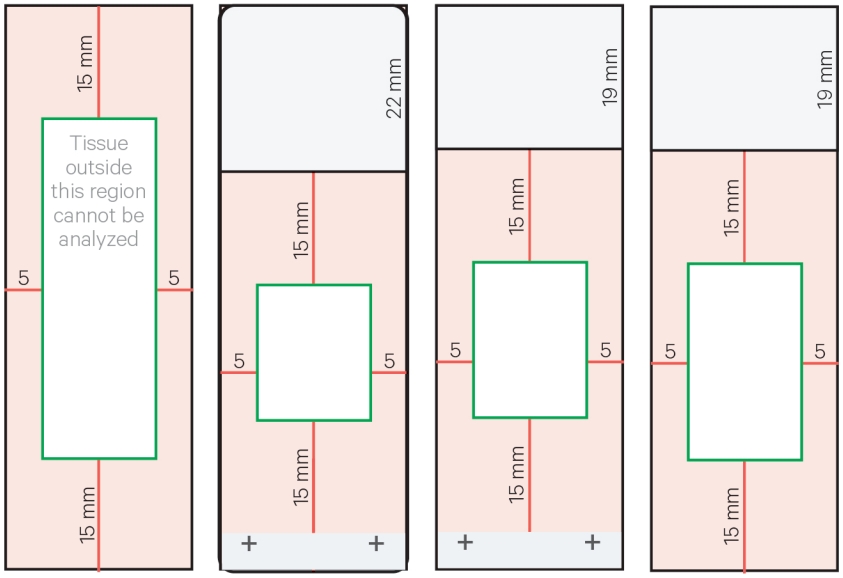
Visium CytAssist Validated Slides

The following slides have been validated for use with the Visium CytAssist instrument.

Item	Length (mm)	Width (mm)	Thickness (mm)	Ground Corners
Epredia Shandon SuperFrost	75.0	25.0	1.0	No
Fisher SuperFrost/ColorFrost	75.0	25.0	1.0	Available as either
Sigma-Aldrich Poly Prep Slides	75.0	25.0	1.0	No
VWR SuperFrost Plus	75.0	25.0	1.0	No

If unsure of slide part number, refer to "blank slide" diagram below for general guidance. Diagrams for verifying that tissue sections are placed in the allowable area can also be found in the Visium CytAssist Quick Reference Cards - Accessory Kit (Document CG000548).

While slides are specified as being 25 mm x 75 mm, manufacturing tolerances may lead to dimensions that are too small or large to be compatible with 10x Genomics products. Tissue Slide dimensions must be within 24.8 mm - 25.3 mm in width and 74.4 mm - 76.2 mm in length to fit the Visium CytAssist Tissue Slide Cassette. Minimum slide dimensions: 24.8 x 74.4 mm. Maximum slide dimensions: 25.3 x 76.2 mm.



Step 1:

Training Step 1

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

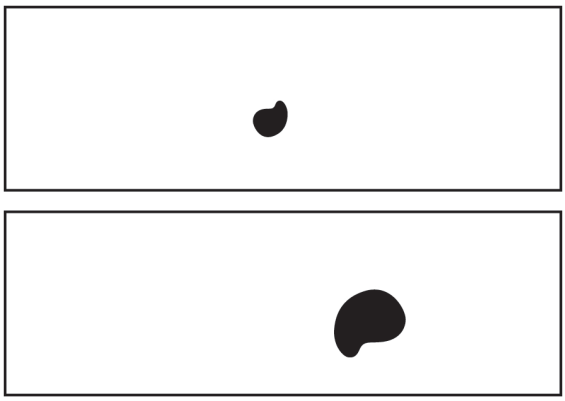
1.1 Draw Tissue

This step is unnecessary if performing the training workflow with eosin stained tissue sections on glass slides.

a. Overlay glass slides over the diagram on the Tissue Slide Alignment (1 of 2) page of the Visium CytAssist Accessory Quick Reference Cards (Document CG000548). The tissue should lie within the green allowable area:

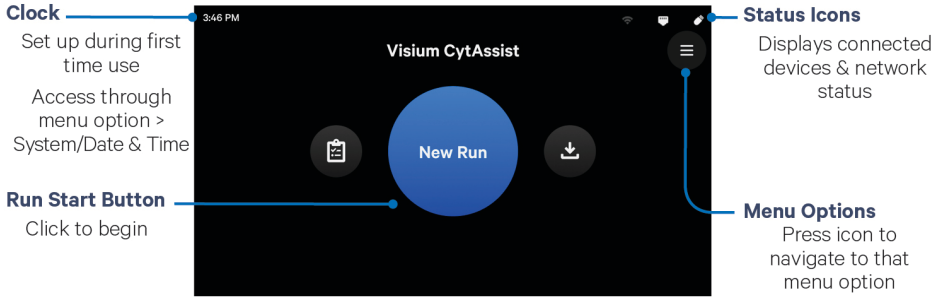
- 15 mm from top and bottom edges
- 5 mm from the sides

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.

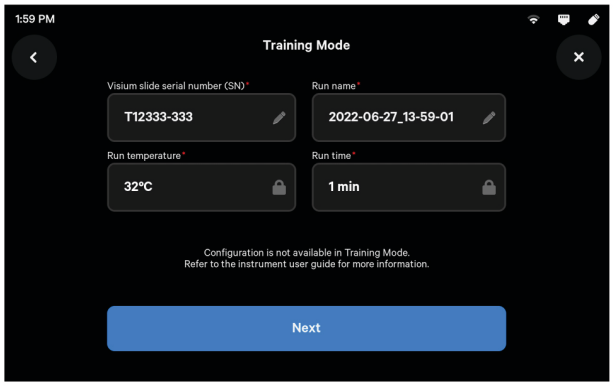


a. Press blue "New Run" Button on the touchscreen to initiate run.

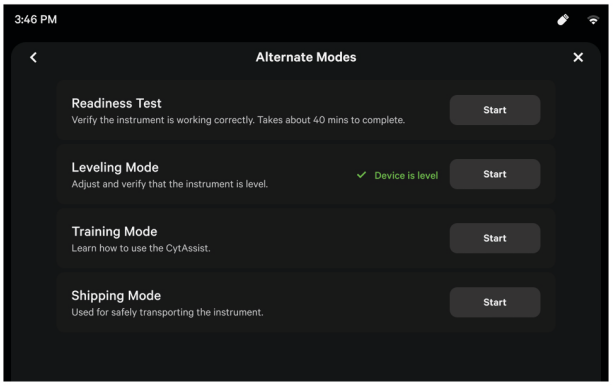
b. Enter new run information, including:

- 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.



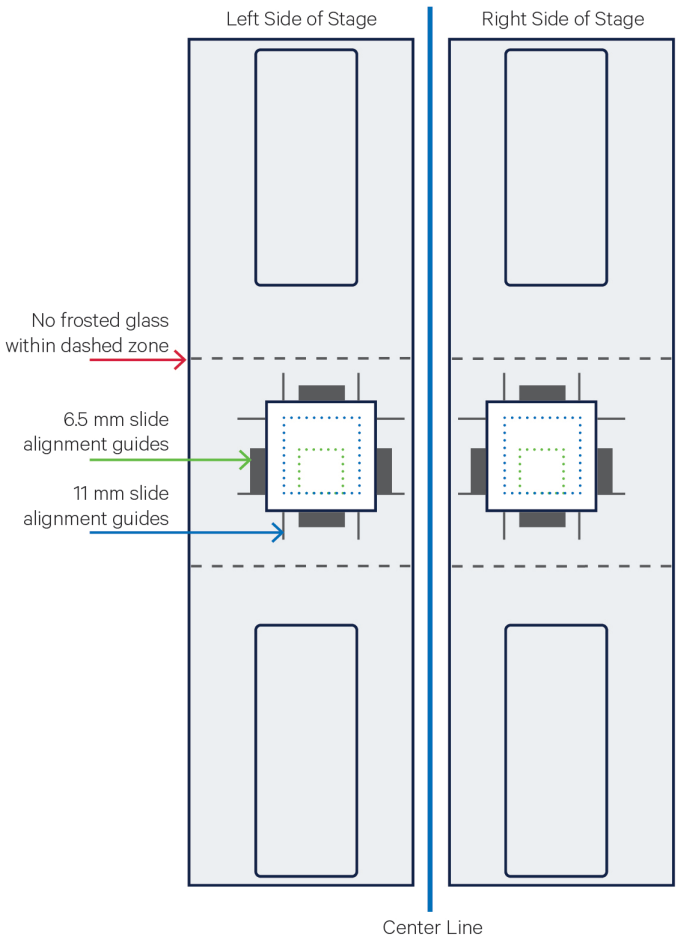
The Visium CytAssist may also enter training mode via the Alternative Modes menu. To access the Alternative Modes Menu, press the menu icon on the home screen, then press "System".



1.3 Position Tissue Slide on the Tissue Slide Stage

a. After entering the slide serial number, the instrument lid will unlock. Open the lid and ensure the tissue on the slide fits within the allowable area of the Tissue Slide Stage.

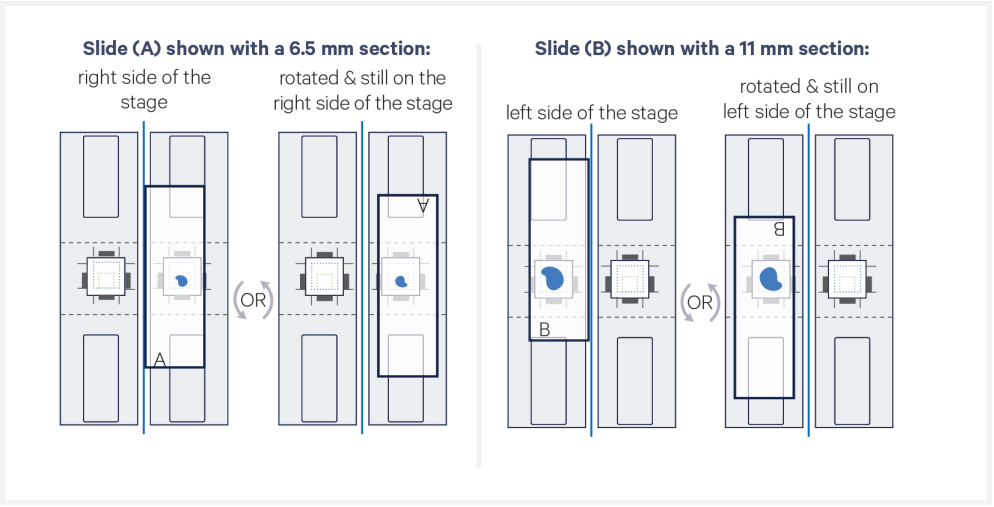
Align tissue to the center of the alignment guides for 6.5 mm (rectangles) or 11 mm (lines) capture areas on either the left or right side of the stage. Align the center of the tissue region of interest to the center of the capture area, rather than aligning the edge of the region to the edge of the capture area.



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.

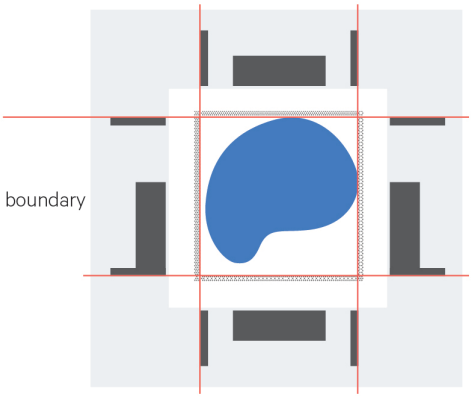
b. If necessary, rotate the slide 180° as shown to better place off-center tissues closer to the center line. Slides should not overlap the center line.



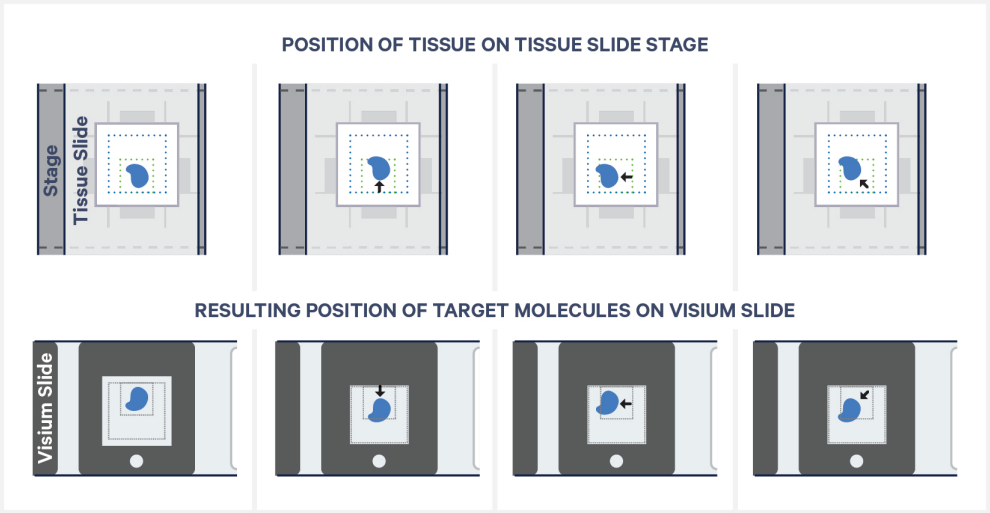
Overlay of a 11 mm fiducial frame and the alignment guides

Note that the **outside** of the alignment guides mark the boundary of the fiducial frame on a Visium CytAssist Slide capture area.

When placing a tissue slide on the Tissue Slide Stage, tissue may come up to the edge of the 11 mm alignment guides.



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.


POSITION OF TISSUE ON TISSUE SLIDE STAGE
Stage
Tissue Slide
RESULTING POSITION OF TARGET MOLECULES ON VISIUM SLIDE
Visium Slide

Step 2:

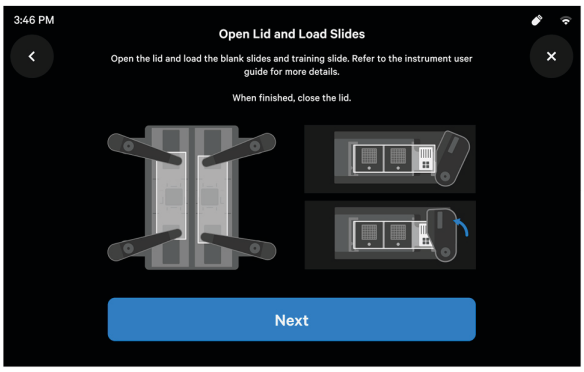
Training Step 2

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 Tissue Slides onto the Tissue Slide Stage

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



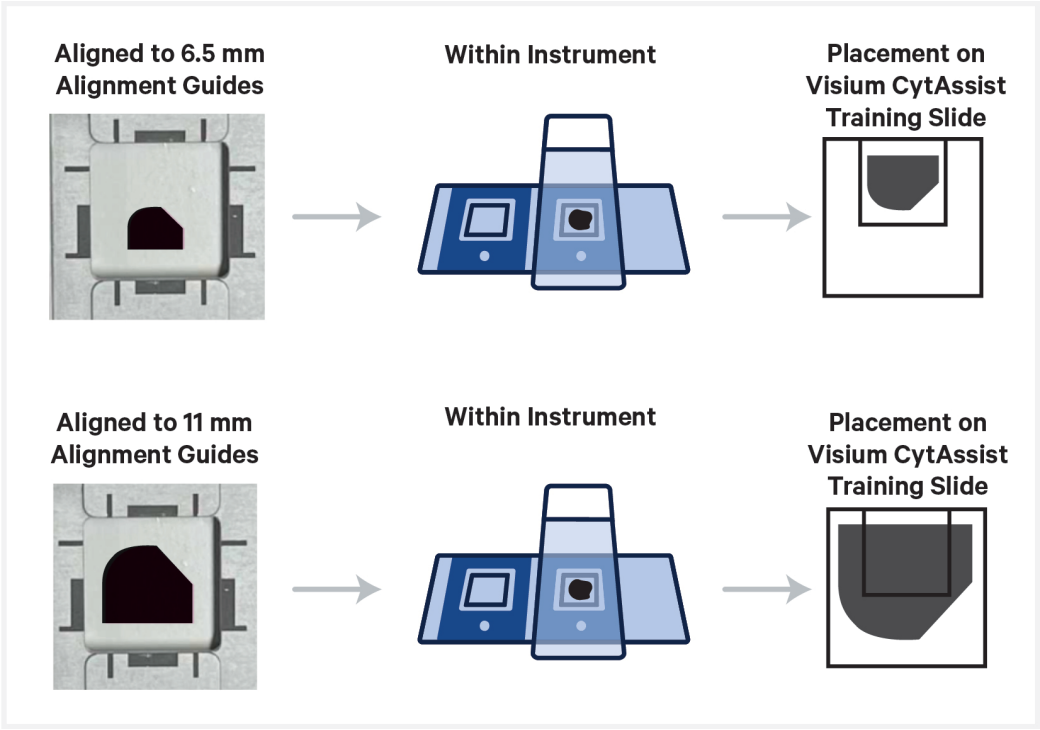
b. Wipe the back of the Tissue Slides with a laboratory wipe.

c. Press down on the wide end of clip to lift and pivot the narrow end of the clip. Move the clips to their outermost position.

d. Use one hand to hold the slide in place and the other to pivot the clips and overlap the slide. **Slide may be held in place with only one clip.**

e. Lay the tissue slide flat against the stage surface with the tissue facing away from the instrument. Use fingers to position the mock tissue or eosin stained tissue within the center of the alignment guides.

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



2.2 Run Visium CytAssist

a. Load Visium CytAssist Training Slide.

b. Add **75 µl** nuclease-free water to 1.5 ml microcentrifuge tube.

c. Centrifuge tube for 5 sec. Centrifugation is meant to mimic steps performed in the CytAssist assay.

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

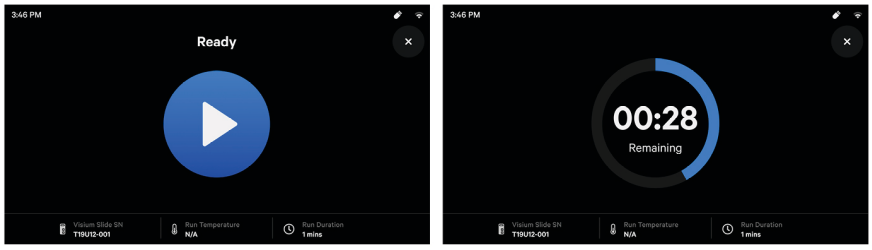
The time between the addition of water and starting the Visium CytAssist training run should be less than **5 min**.

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 support data.

i. Remove the Visium slide from the instrument and proceed to data export. 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.

Step 3:

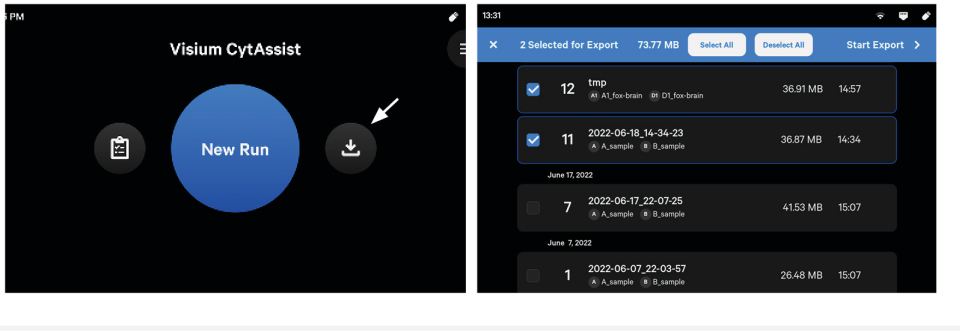
Training Step 3

3

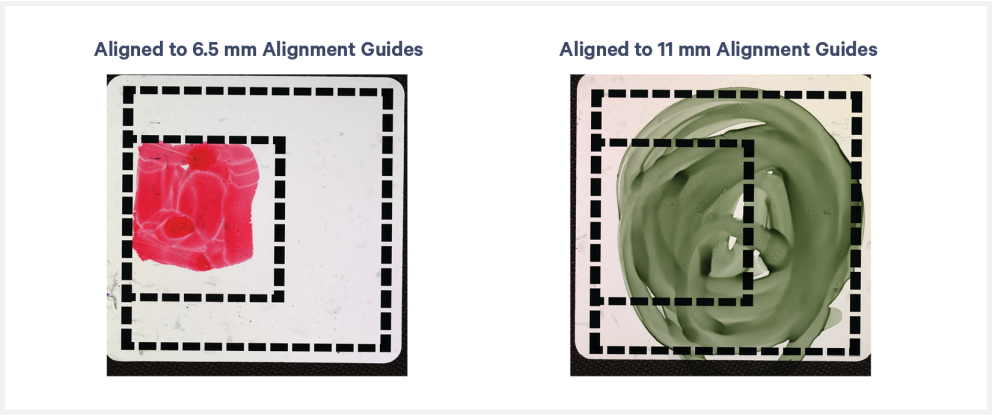
3.0 Visium CytAssist Image Review

Data occurs in the log page - it 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.

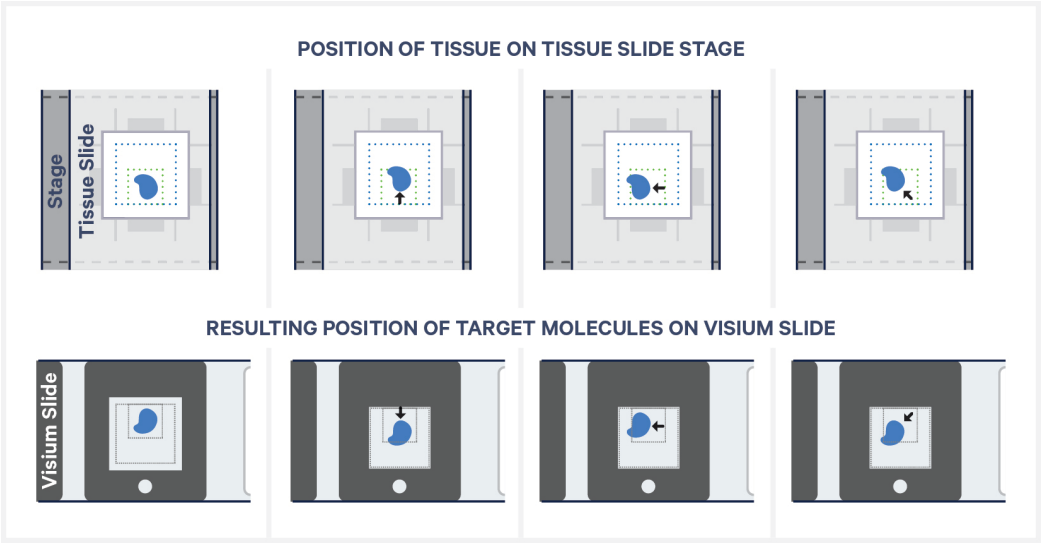
a. Once run is complete, export images via USB using the export button on the home screen. Follow on-screen instructions. Data export will take approximately **2 min**. Refer to Visium CytAssist Instrument User Guide (CG000542) for more information.



b. Assess accuracy of tissue alignment. If alignment was successful, the drawn tissue section should appear within the desired (6.5 mm or 11 mm) frame. Image should be free of artifacts such as bubbles.



Refer to the diagram below for information on how to move the tissue slide to correct alignment.



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

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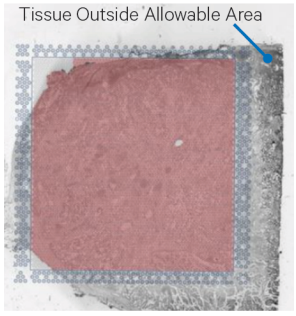
Troubleshooting

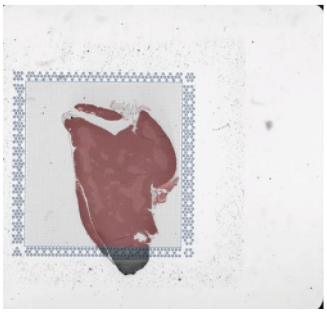
Tissue Not Within Allowable Area

Tissue Outside of Allowable Area is Not Analyzed

Tissue Larger than Capture Area



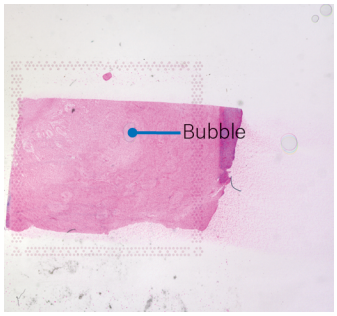
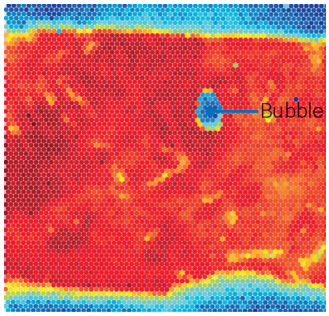
Tissue Not Properly Aligned



Tissues that are not placed within the allowable area on approved 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 into the CytAssist instrument.

Bubbles Trapping During CytAssist Enabled RNA Digestion and Tissue Removal

Bubbles may Result in no Usable Sequencing Reads during the CytAssist Assay

Eosin Stained Tissue	UMI Counts
 Some eosin may be washed off during an instrument run, as shown on the right side of this image. This does not affect performance.	

The images above show the effect of bubble generation on downstream UMI counts during the actual Visium CytAssist assay. 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.