10x GENOMICS

User Guide | CG000542 | Rev A

Visium CytAssist

with Readiness Test

For use with:

Visium CytAssist & Accessory Kit, 12-Month Warranty, PN-1000441
Visium CytAssist Instrument Accessory Kit, PN-1000433

Visium CytAssist & Accessory Kit, 24-Month Warranty, PN-1000442
Visium CytAssist Instrument Accessory Kit, PN-1000433

Notices

Document Number

CG000542 | 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. In the event of an Instrument failure resulting from an update, such failed Instrument will be replaced or repaired in accordance with the 10x Limited Warranty, Assurance Plan or service agreement, only if such Instrument is covered by any of the foregoing at the time of such failure. Instruments not covered under a current 10x Limited Warranty, Assurance Plan or service agreement will not be replaced or repaired.

Support

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Document Revision Summary

Document Number

CG000542 | Rev A

Title

Visium CytAssist Instrument User Guide with Readiness Test

Revision

Rev A

Revision Date

July 2022

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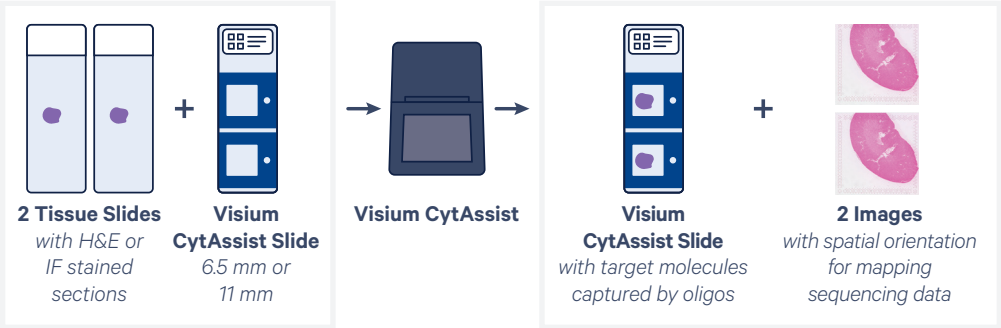
Introduction

Introduction

The Visium CytAssist is a compact, bench top instrument that automates the transfer of transcriptomic analytes from standard microscope slides to Visium slides, enabling spatial profiling insights from more samples. Compatible with hematoxylin and eosin (H&E)- or immunofluorescence (IF)-stained FFPE tissue sections, the CytAssist instrument allows pre-sectioned tissues to be used for the Visium workflow and eliminates the need to directly section onto Visium slides.



Further optimization of Visium experiments can be achieved by pre-screening tissue sections using standard histological techniques to find biologically significant sections, then using the CytAssist instrument workflow to precisely align those sections within the Visium Capture Areas. Using Visium Cytassist, analytes from up to two FFPE tissue sections can be captured on CytAssist specific slides in less than one hour per run.

Highlights

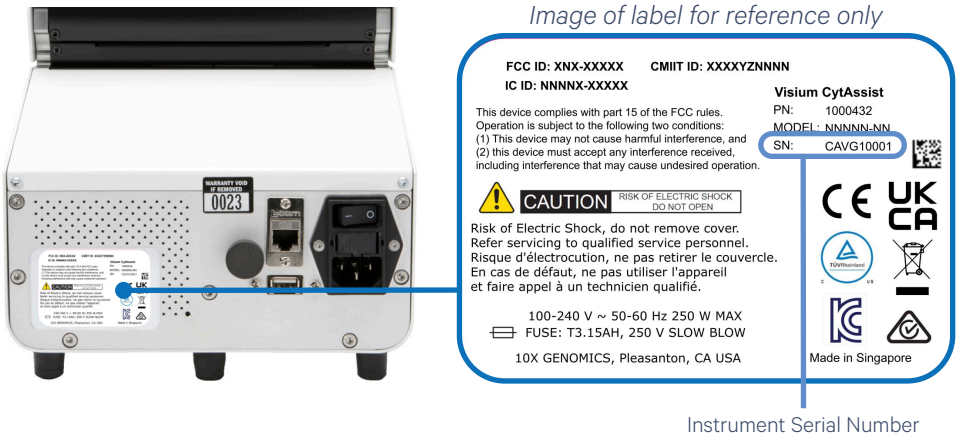
- Simplify sample handling with automated transfer of transcriptomic analytes from standard slides onto the Capture Area of a Visium slide
- Eliminate the need to section directly onto the Visium slide, and expand sample compatibility to pre-sectioned slides
- Maximize insights from Visium experiments by pre-screening tissue sections with standard histological techniques to select the most biologically significant sections
- Precisely capture analytes from up to two FFPE tissue sections per run in less than one hour using CytAssist specific slides and reagents

Product Compatibility

The Visium CytAssist is designed for compatibility with the Visium CytAssist Spatial Gene Expression for FFPE assay:

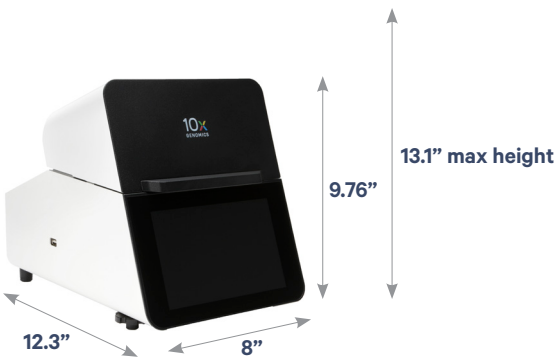
- For Mouse or Human tissues
- With 6.5 mm or 11 mm Capture Area options

Product Identification Label



Product Specifications

Parameter	Specifications
Weight	~18.3 lb (8.3 kg)
Instrument Dimensions with: Lid closed Lid open Maximum height	W D H 8”[203 mm] × 12.3”[313 mm] × 9.76”[248 mm] 8”[203 mm] × 12.3”[313 mm] × 12.3”[312 mm] 13.1” [334 mm]
Electrical Requirements Nominal *from a standard 3-prong wall receptacle that includes a safety ground pin*	100-240 VAC, 50/60 Hz, 250 W 80-264 V operational range (+/- 10% of nominal) Over voltage Category II (standard receptacle)
Pollution	Degree 2 (Indoor Use Only)
Ventilation Requirement	Minimum 4” [10 cm] Around all sides
Operating Temperature	64-82°F [18-28°C] Use in a typical indoor laboratory environment. Extreme temperature conditions will affect the sensitive reagents used with the instrument.
Humidity	30-80% R.H. non-condensing
Power Cable Length *Power cables will be in accordance with regional specifications*	6-7 ft [2 m] Standard



The instrument requires a **~20 min cool down** between runs.

Safety & Compliance Information

Safety

Before operation, ensure that all potential users have received:

- Instruction in general safety practices for laboratories.
- Instruction in specific safety practices for the instrument.
- All related Safety Data Sheet (SDS) documents.

Precautions are illustrated in the following way:

Symbols	Description
	The general Warning symbol indicates the possibility of damaging the instrument or compromising the results of a method.
	The Electrical Hazard symbol indicates the presence of electrical components that can be harmful to the operator if handled incorrectly.
	The Mechanical Hazard symbol indicates the presence of moving mechanical parts that can be harmful to the operator if handled incorrectly.
	The Hazardous Materials symbol indicates the presence of materials that are toxic or otherwise harmful to the operator if handled incorrectly.
	The Biohazard symbol indicates the presence of biological samples that can be harmful to the operator if handled incorrectly.

Ensure ground is reliably connected before plugging the instrument's power cord into the power source (receptacle). *Grounding is required to prevent electric shock. If the power source is not grounded, qualified personnel must first install a reliable safety ground.*

Pinch risk: Ensure no obstructions or fingers present near closing lid.

Warning: Avoid using the Visium CytAssist in a manner not specified by 10x Genomics. *The Visium CytAssist has been designed to protect the user. If used improperly, the intended user protections can be impaired.*

Regulatory

The Visium CytAssist has been designed, tested, and certified to be in compliance with the following standards:

Certification	Standards
	TUV Certification only for Visium CytAssist UL 61010-1:2012 and CAN/CSA C22.2 No. 61010-1-12 with a cTUVus mark to indicate that the product has been tested and certified to Canadian and US standards by TUV Rheinland and can be legally installed in those countries.
	IEC/EN 61010-1:2010 (3rd Edition): Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory use.
	EN 61326-1:2013: Electrical Equipment for Measurement, Control and Laboratory Use. EMC Requirements.
	The RCM mark indicates an electrical product complies with all the requirements of the electrical and EMC regulations of Australia and New Zealand in accordance with AS/NZS Standards
	CE Mark indicates that assembly is covered by a Declaration of Conformity, and has been declared in conformity with the provisions of all applicable directives in the European Union.
	UKCA Mark indicates that assembly is covered by a Declaration of Conformity, and has been declared in conformity with the provisions of all applicable directives in the United Kingdom.
	EN 61326-2-6: Specifies minimum requirements for immunity and emissions regarding electromagnetic compatibility for in vitro diagnostic medical equipment, taking into account the particularities and specific aspects of this electrical equipment and their electromagnetic environment.
	EN 61000-3-2: Electromagnetic compatibility (EMC) - Part 3-2: Limits - Limits for harmonic current emissions (equipment input current ≤16 A per phase).
	EN 61000-3-3: Electromagnetic compatibility (EMC) - Part 3-3: Limits - Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems, for equipment with rated current ≤16 A per phase and not subject to conditional connection.
	RoHS Directive (2011/65/EU) and amendment (EU) 2015/863: Restriction of the use of certain hazardous substances in electrical and electronic equipment.
	WEEE Directive (2012/19/EU): Waste Electrical and Electronic Equipment.
	FCC ID: N6C-SDPAC, IC: 4908A-SDPAC FCC Part 15 Class A. NOTE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense. This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.
	ICES-003 (Canada): This Class A digital apparatus complies with Canadian ICES-003. Cet appareil numérique de la classe A est conforme à la norme NMB-003 du Canada.

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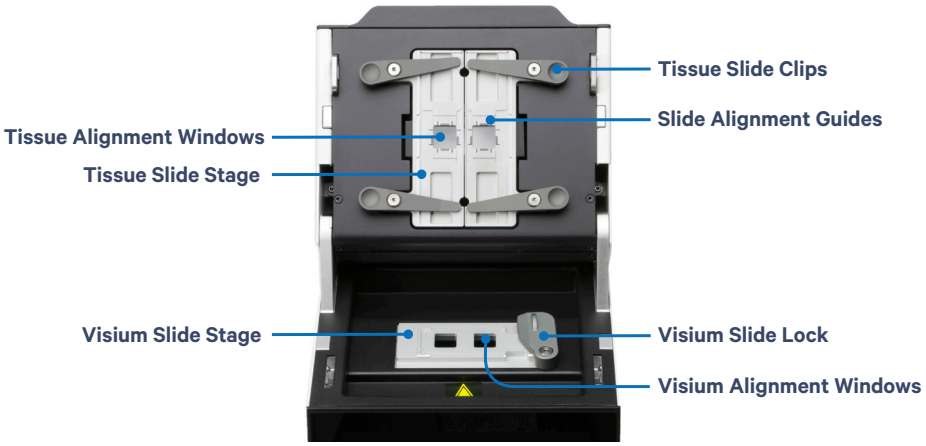
System Components

System Components

Exterior

Interior



Back

Accessory Kits

Visium CytAssist Instrument Accessory Kit PN-1000433

Product	#	Part Number (Item)
Universal Power Cord, 10 Amp-250 V *(IEC 60320 C-14, with C-13 Detachable Adapters)*	1	3000603
USB, 8 GB *The Visium CytAssist instrument is compatible with VFAT and exFAT formatted USBs.*	1	3000664
Visium CytAssist Quick Reference Cards – for Accessory Kit Instruction	1	CG000548
Visium CytAssist Training Kit	**1**	**1000458**
Visium CytAssist Training Slide Assembly	2	2000681
Blank Slides	2	3000868
Visium CytAssist Calibration Kit	**1**	**1000459**
Visium CytAssist Calibration Slide Assembly	1	2000680
Blank Slides	2	3000868

Additional Items

Product	#	Part Number (Item)
Shipping Bolt *Located in the back of the instrument during transportation; removed during installation and stored apart from the instrument*	1	3000986

Tips & Best Practices

Icons

TIPS

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

Check Tissue Placement on Slide

Check Slide Dimensions

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 Visium CytAssist. 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**

Check Tissue Placement on Slide

Ensure that the slide surface with the tissue is facing the user.

Use this section to determine if the tissue section is located in an area that results in successful analyte transfer and imaging. The Visium CytAssist Quick Reference Cards – for Accessory Kit Instruction (CG000548), located within the Instrument Accessory Kit (laminated document with concise instructions).

a. Ensure that the tissue region of interest will fit within the Capture Area of a Visium CytAssist Spatial Gene Expression Slide. Overlay the slide, centering the tissue on either the 6.5 mm or 11 mm square.

b. <u>If the slide has no frosted areas</u>, overlay on this diagram. The tissue should lie within the green allowable area:

- **15 mm** from the short sides
- **5 mm** from the long sides

If printing this page:

Select "actual size" or "100%" to print to scale. The slide diagram should measure 25 mm x 75 mm.

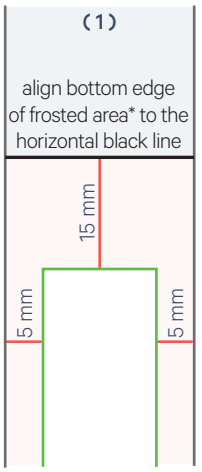
Check Tissue Placement on Slide *contd.*

If the slide has a frosted end or frosted marks along the bottom, overlay on diagram 1 and then on diagram 2.

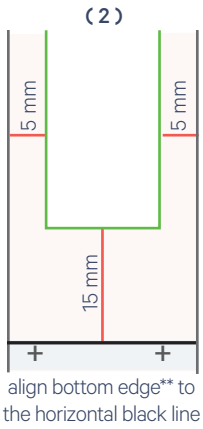
Due to variability in the dimensions of frosted areas across slide brands, the allowable area for tissue placement is also variable across slide brands.

Check the allowable area from both the top (1) and bottom (2) to ensure the tissue lies within the green allowable area:

- **15 mm** from edge of frosted area/marks
- **5 mm** from the sides



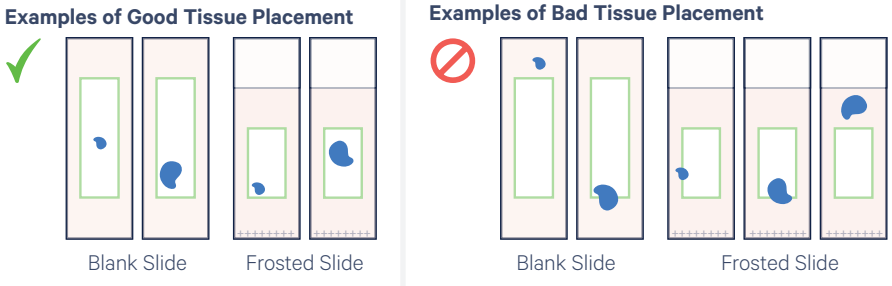
**If text is present below frosted area of slide, align bottom of the letters to the line*



***If markings are present at bottom edge of slide, align markings to the + signs*

If printing this page:
Select "actual size" or "100%" to print to scale. The slide diagrams should each measure 25 mm wide.

c. Refer to the examples of good and bad tissue placement.



Refer to the Visium CytAssist Training Kit User Guide (CG000549) for further guidance.

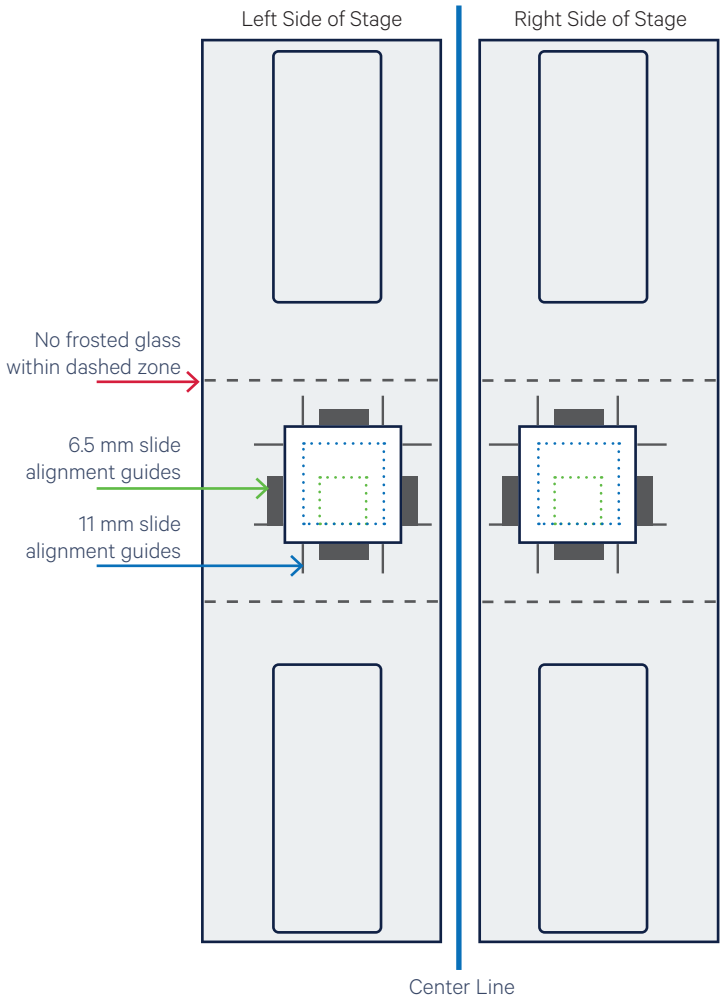
Tissue Slide Placement on Tissue Slide Stage

Position Tissue Slide on the Tissue Slide Stage

Use this section to determine if the tissue section is located in an area that results in successful analyte transfer and CytAssist-mediated imaging. The Visium CytAssist Quick Reference Cards – for Accessory Kit Instruction (CG000548), located within the Instrument Accessory Kit, contain a concise and cleanable version of this content.

d. Ensure the tissue on each slide fits within the allowable area of the Tissue Slide Stage.

e. 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.



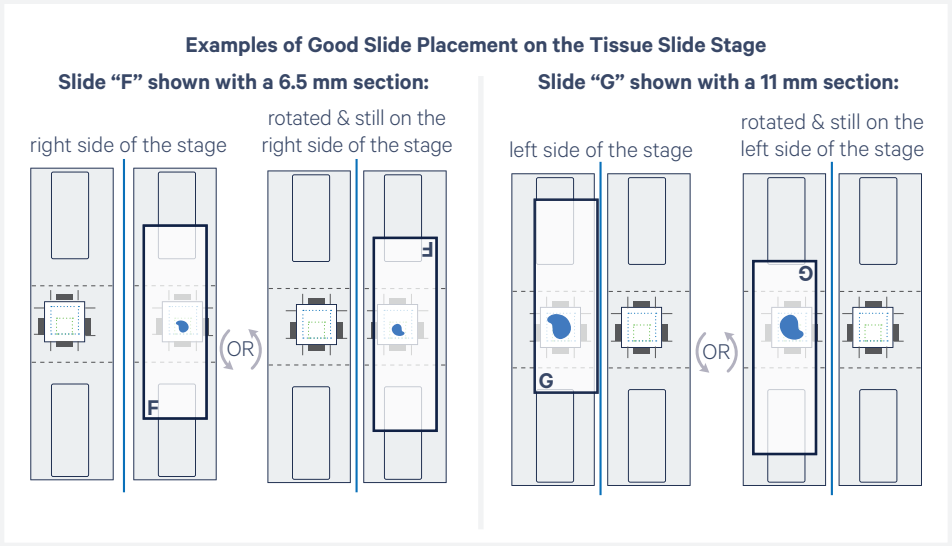
The dotted outlines of the Capture Areas that are shown in diagram are not present on the instrument's surface.

Slides should not overlap the center line.

If printing this page:
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.

Tissue Slide Placement on Tissue Slide Stage *contd.*

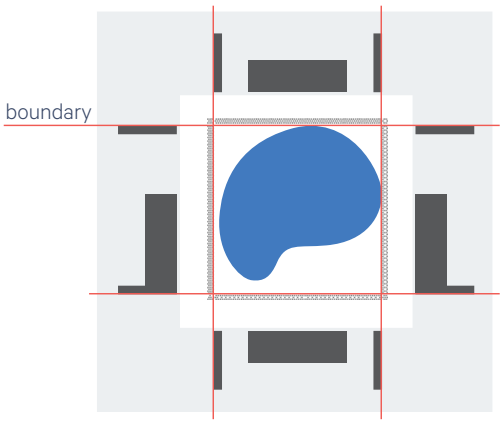
f. If necessary, rotate the slide 180° as shown to better place off-center tissues closer to the center line. Slides should not cross/overlap the center line.



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.



Refer to the Visium CytAssist Training Kit User Guide (CG000549) for further guidance.

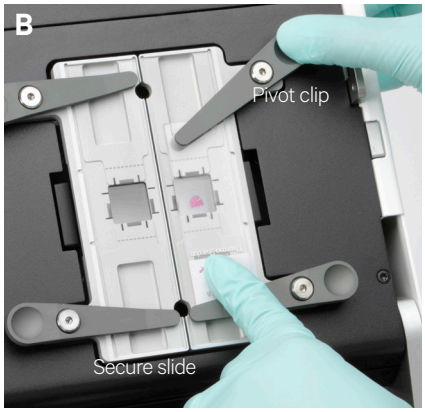
Load Tissue Slides onto the Tissue Slide Stage

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

Tissue Slide Placement on Tissue Slide Stage *contd.*

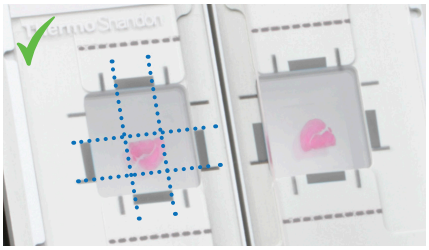
b. Lay the tissue slide flat against the stage surface. Both hands needed: use one hand to hold the slide in place and the other to pivot the clips and overlap the slide. Ensure that at least one clip secures the slide before adjusting the position further.

c. Use fingers to finely adjust the position of the tissue within the alignment guides. DO NOT touch the tissue section.



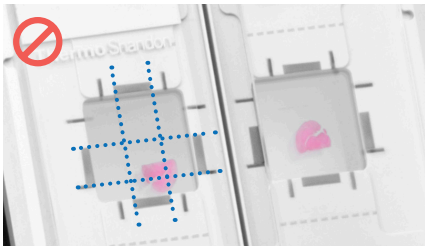
CORRECT

Tissue lies completely within alignment guides



INCORRECT

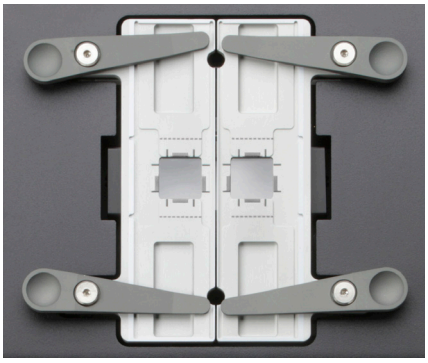
Tissue is intersected by alignment guides



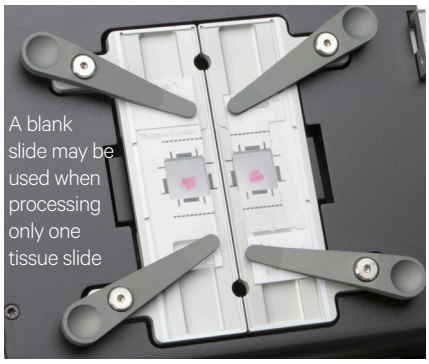
6.5 mm Capture Area used as an

d. If only one tissue on one slide will be loaded, use a blank slide for the second position on the Tissue Slide Stage.

Tissue Slide Stage is Empty



Tissue Slide Stage is Loaded Correctly with Two Slides



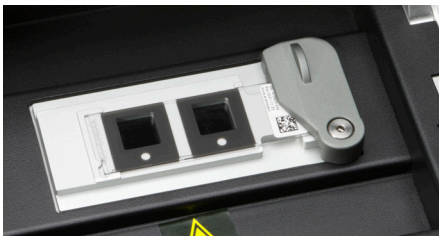
Visium Slide Placement on Visium Slide Stage *contd.*

e. Secure the slide by pivoting the Visium Slide Lock gently to the left, ensuring that the slide is not impacted. The lock will partially obscure the slide label when correctly secured.

f. Secure the slide by pivoting the Visium Slide Lock gently to the left, ensuring that the slide is not impacted. The lock will partially obscure the slide label when correctly secured.

Visium Slide Stage is Empty

Visium Slide Stage is Loaded Correctly with One Slide

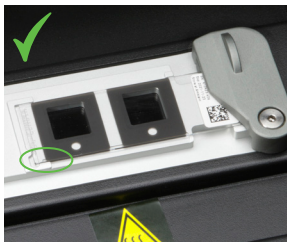


CORRECT

Slide fits inside the grooves.

Label on the right is partially obscured by the lock.

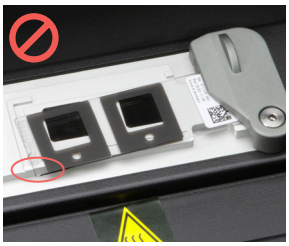
Capture Areas line up with alignment windows.



INCORRECT

Slide does not fit inside the grooves.

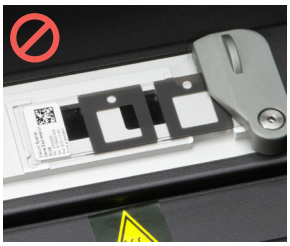
Capture Areas do not line up with alignment windows.



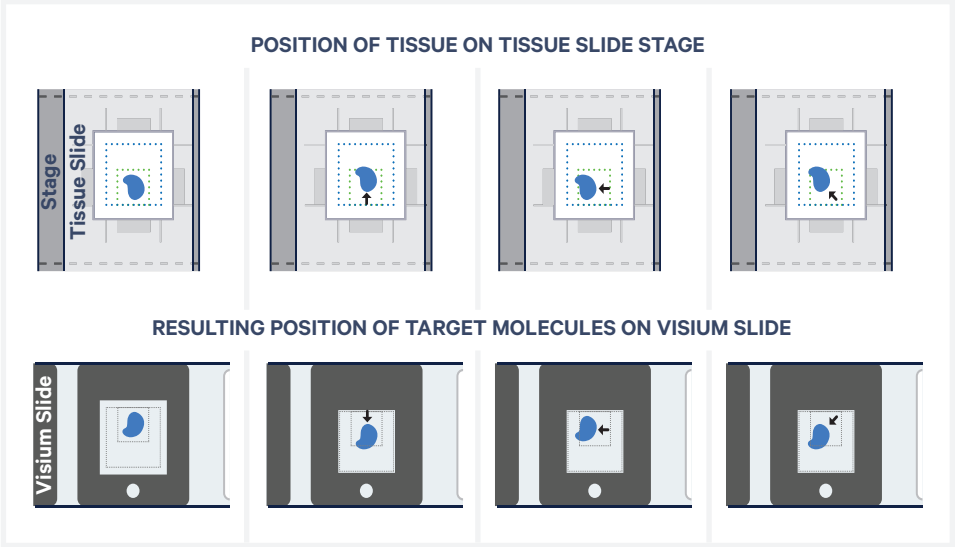
INCORRECT

Label is on the left.

Capture Areas do not line up with alignment windows.



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.

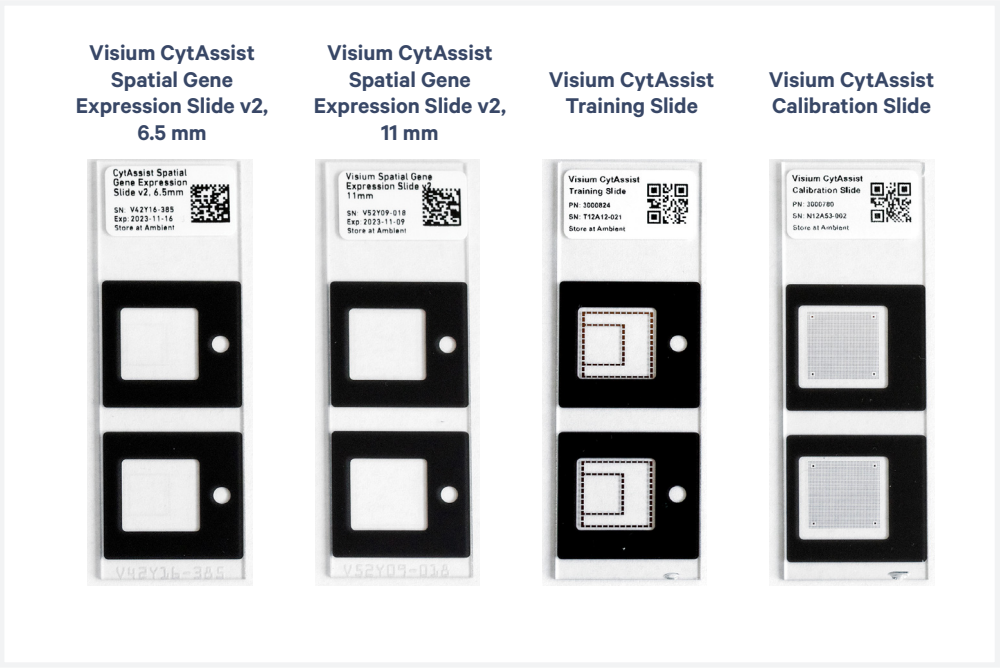


Visium Slide Placement on Visium Slide Stage

Visium Slides

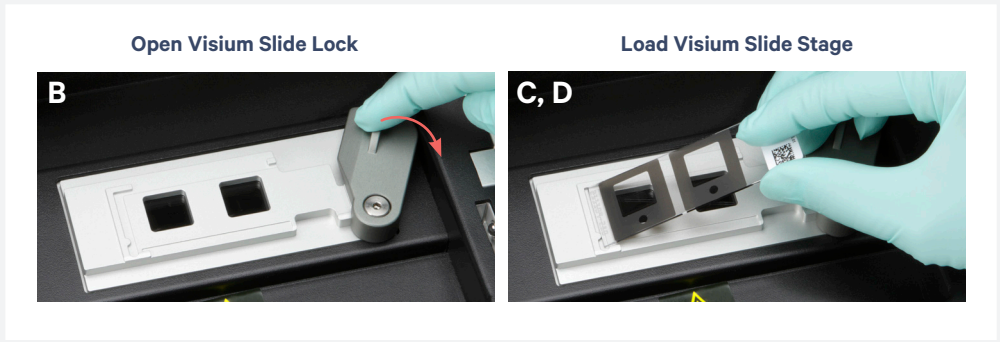
The Visium Slide Stage holds one of the following at a time:

- Visium CytAssist Spatial Gene Expression Slide v2, 6.5 mm
- Visium CytAssist Spatial Gene Expression Slide v2, 11 mm
- Visium CytAssist Training Slide
- Visium CytAssist Calibration Slide



Load Visium Slides onto the Visium Slide Stage

a. Inspect the Visium Slide Stage for dust and debris, and clean according to the Maintenance section, as needed.



b. Open the Visium Slide Lock by using one finger to pivot.

c. Line up the slide with the label face up and oriented to the right.

d. Fit the slide within the raised grooves on the left, top and bottom.

10x Genomics Cloud

10x Genomics Cloud (accessible through cloud.10xgenomics.com), allows creation of an universal user account for all of the web services offered by 10x Genomics. Using the instrument management tool, users can access the instrument information and download the latest firmware updates for their instruments.

Each Visium CytAssist instrument is required to be registered to the 10x Genomics Cloud during the First Time Use (FTU) set up process. Users may register multiple instruments to their accounts, and each instrument can optionally be registered to multiple 10x Genomics accounts. Instruments connected to the network can also transfer serviceability data directly to 10x Genomics, enabling streamlined customer service.

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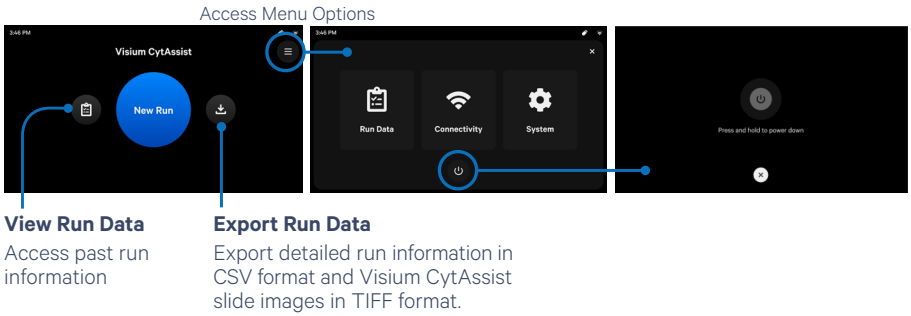
Menu Options

Menu Options

The instrument home screen enables starting a run, monitoring run progress, and accessing the Menu Options (top right corner of the screen) for Logs, Connectivity, and System information.



The instrument's screen will dim after a few minutes of inactivity. Power off the instrument by tapping the power icon at the bottom of the menu options screen. The system will go into a soft power down (screen is dimmed and displays time and name of instrument).

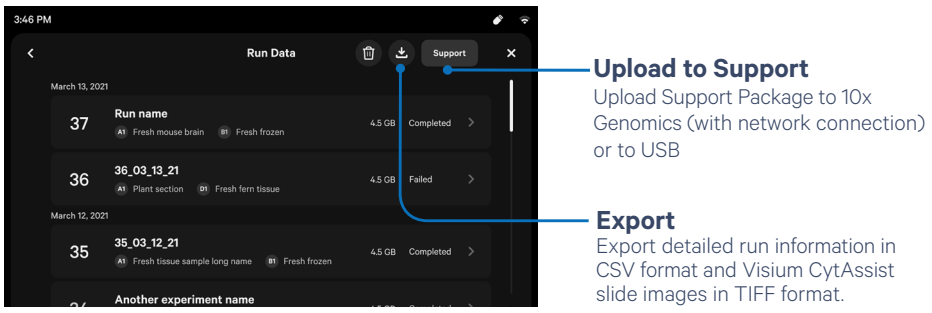
To shut down the instrument completely, place the instrument in the soft power down state and then flip the power switch at the back (no preliminary steps needed before a complete shutdown). See Moving & Shipping the Instrument section.

Instrument Data

From this menu option, access and export:

- Run Data - includes CytAssist images, run csv with metadata (such as sample, run name, slide serial number etc.); Size ~10s of mb/run
- Support Package - includes Run Data plus instrument telemetry and sensor information (captured both during instrument run and instrument idle* time); Size ~100s of mb/run

Share Support Package with 10x Genomics Technical Support for troubleshooting purposes. Click an entry for more details.



*Idle time data are only instrument-specific logs that are not related to runs. The data are exported with the Support Package. During First Time Use (FTU) set up, it can be accessed using the help icon on the top right corner of the screen.

Menu Options
contd.

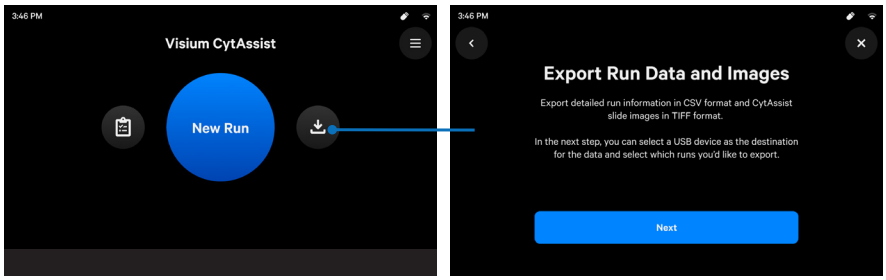
Data export ranges in size from 10s to 100s of MB, depending on how many runs are selected. If Automatic Upload is turned on, instrument and system data are automatically exported or uploaded after a run but the CytAssist images and Support Packages are not.

The instrument file stores up to 100 Run Data and up to 10 Support Data. The instrument does not automatically delete Run Data without user permission while older Support Data is deleted automatically (oldest files are deleted first).

It is critical that users proceed to the next stage of the workflow in the assay kit user guide immediately, rather than exporting data right away.

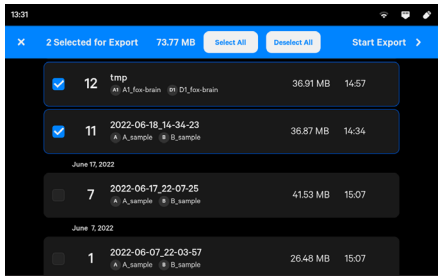
Export Procedure

a. Click the Download button on the home screen.



b. Insert a USB. Onscreen, select the USB destination for exported data.

c. Select runs for export, then click Start Export. A progress bar will indicate completion.



d. User will be redirected back the Run Data log screen.

Alternatively, clicking on the Run Data icon on the left side of the home screen allows the user to view runs first and click Download later from the upper right hand corner.

Menu Options
contd.

To review service data with 10x Genomics Technical Support:

- Email support@10xgenomics.com with any applicable error codes.
- Upload pertinent logs via USB (no network connection) or directly to 10x Genomics Cloud with a network connection.
  Support personnel can guide the user through these steps.

Connectivity

Configure parameters for Wi-Fi, and Ethernet from this menu option. Note that Wi-Fi configuration may not be available in your region. The minimum requirement is 5 GHz (2.5 GHz not compatible). Ethernet is recommended for optimal performance.

Click Automatic Uploads to enable upload of log files to 10x Genomics Cloud (only instrument data uploaded, not assay images)

Internet connectivity is not required, but is highly recommended so that the instrument may receive security/firmware updates and automatically upload system log files to the 10x Genomics Cloud. 10x Genomics has no remote access to the instrument.

Users may separately configure connectivity settings related to firmware updates and log upload behavior.

Network & Security

- Allow the following outbound ports for 10x Genomics Cloud access: TCP port: 443
- As an additional (optional) level of security, put the instrument on its own network VLAN with Internet-only access.

System

The system runs on a Linux platform. The instrument's hardware and software have been securely implemented to prevent modification and to only execute trusted 10x code. There is no end-user configuration or access to the internal hardware, OS, or applications.

In the System menu:

Locate information about this instrument such as serial number, instrument ID, and firmware version.

Change the date and time by country or time zone. Time can be set automatically via network connection. Toggle between 12-hour or 24-hour time.

Launch Shipping Mode (for safe instrument transportation) through the Alternate Modes menu option. See the Maintenance section, Moving & Shipping the Instrument, for specific instructions on packaging the instrument.

Firmware update notifications will appear on the home screen when the instrument is connected to a network. Alternatively, navigate to cloud.10xgenomics.com to view firmware updates and download via USB.

Firmware Update Procedure

Review the firmware update procedure below:

Step 1: Download and install the update

Procedure with a network connection, auto-download enabled:

e. A pop-up on the home screen will alert the user of available updates.

f. Open pop-up and click to install.

Procedure with a network connection, auto-download disabled:

a. A pop-up on the home screen will alert the user of available updates.

b. Open pop-up and click to download.

c. Click to install.

Procedure with no network connection:

a. Visit cloud.10xgenomics.com to view firmware updates.

b. Download the desired updates onto a USB.

The Visium CytAssist instrument is compatible with VFAT and exFAT formatted USBs.

c. Insert USB into the back of the instrument.

d. Onscreen, navigate through the menu to System > Firmware Update > Update via USB.

e. Click on "Firmware update available."

f. Click to download the update to the instrument.

g. Click to install.

h. After installation is complete, remove USB from instrument.

Firmware Update Procedure *contd.*

Step 2: Reinitialize the instrument

After installing an update, run a Readiness Test. See Readiness Test section for further guidance.

a. To initiate a Readiness Test, navigate through the menu to System > Alternate Modes > Readiness Test. OR press the blue Run Start Button on the touchscreen. **Enter the serial number located on the Visium CytAssist Calibration Slide label.**

b. Locate two blank slides and the Visium CytAssist Calibration Slide in the Instrument Accessory Kit. Clean with compressed air.

TIPS

c. Follow onscreen instruction and see Tips and Best Practices section to load the blank slides and calibration slide onto the instrument.

d. Press the blue play button to start the test.

e. Upon test completion, follow onscreen instruction to unload slides. Follow onscreen instruction in the event of a failed or incomplete test.

f. Store the blank slides and the calibration slide in their original packaging bag, away from sunlight and extreme temperature. Retain for future use.

g. Wipe down the Tissue Slide Stage and the Visium Slide Stage with 70% ethanol or 70% isopropanol and laboratory wipes. Use compressed air to dry and remove debris as needed.
Clean glass windows with dry polyester swabs (such as Texwipe TX714A Cleanroom Swab, 0.5”L - EW-33677-62 from Cole-Parmer or equivalent) and compressed air. Use a bright light (e.g. flashlight) to inspect the glass windows for any smudges and remove them as described above. See Maintenance section for more details.

h. Close the lid (clicking sound indicates closure). Wipe down the base and lid of the instrument.

i. The instrument is now ready for use.

10x GENOMICS

System Operation

Step 1: Installation

Bench Space

Follow these instructions for installation to operate the Visium CytAssist properly and obtain maximum performance of the system. Before unboxing the instrument choose a location that:

- Is not situated in direct sunlight.
  Direct sunlight leads to inadequate cooling conditions or rapid evaporation of reagents.
- Includes a level, sturdy laboratory bench that is not subject to movement or vibration.
- Provides maximum bench space (front to back).
  Avoid areas that have obstruction in the rear such as power strips or hose lines (spigots for vacuum or air).
- Allows access to the plug at the wall receptacle (to safely disconnect power).
- Allows access to the detachable power cord at the rear of the instrument
- Provides a minimum of 4" (10 cm) clearance around unit to allow free air circulation.

Warning: Avoid using the Visium CytAssist in a manner not specified by 10x Genomics. *The Visium CytAssist has been designed to protect the user. If used improperly, the intended user protections can be impaired.*

General Power Safety

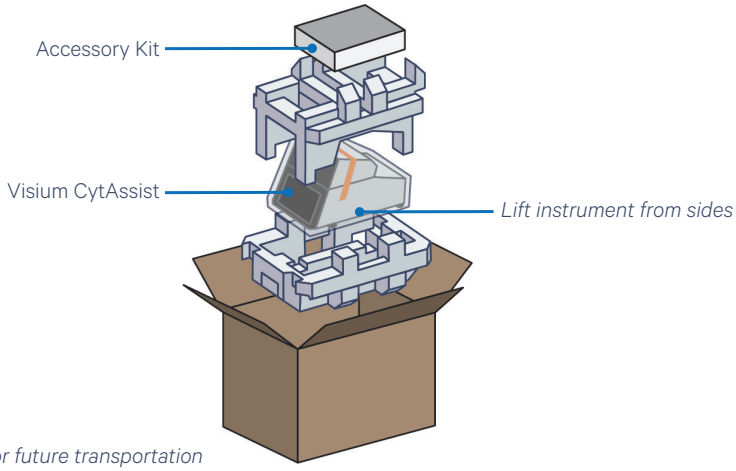
Grounding is required to prevent electric shock. If the power source is not grounded, qualified personnel must first install a reliable safety ground.

- DO NOT plug the instrument power cable into an electrical outlet if the power cable is damaged.
- To prevent electric shock, plug the instrument power cable into properly grounded outlets.
- When using an extension cable or power strip, ensure that the total ampere rating of the instrument does not exceed the ampere rating of the extension cable. The extension cable must be designed for grounded plugs and plugged into a grounded wall outlet.
- Be sure to grasp the plug, not the cable, when disconnecting the instrument from an electric socket.

Step 1: Installation *contd.*

Unbox

DO NOT POWER ON until ready for First-Time Use setup



a. Open box according to box label "this side up."
Carefully cut or remove the tape on the top of the box and open the top flaps. Ensure that cut is not too deep as it may damage the Instrument Accessory Kit box.

b. Remove the Instrument Accessory Kit box, documents, and packaging material.

c. Lift the instrument from the sides. Place on bench top. Carefully remove all packaging materials/tape from the exterior of the instrument. Retain all packaging materials in the event that the instrument needs to be moved (see Moving & Shipping the Instrument section).

d. Remove Shipping Bolt from the back of the instrument. Retain for future use (the Shipping Bolt holds the camera focus in a safe position during transportation).

e. Open lid. Carefully remove tape from the four Tissue Slide Clips and the Visium Slide Lock as well as from the opposite end.

f. Remove glass protective films from the alignment windows on both stages. Close the lid (clicking sound indicates closure).

Remove shipping tape and then glass protective films

Step 1: Installation *contd.*

g. Within the Instrument Accessory Kit box, locate the universal power cord and plug adaptors. Attach the correct country adaptor to the universal adaptor.

h. Plug the Visium CytAssist into a properly grounded outlet of proper voltage (see Product Specifications). If using an Ethernet cable (not provided) to connect to the network, connect cable directly to the instrument.

DO NOT power on yet.

Ensure power source is reliably grounded

Step 2: First-Time Use

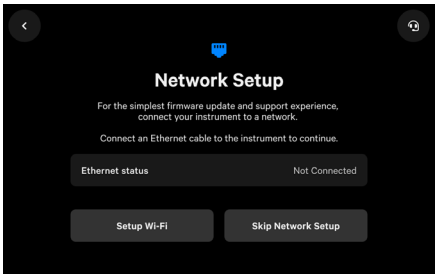
The instrument will walk through a first-time setup protocol. Setup requires the following:

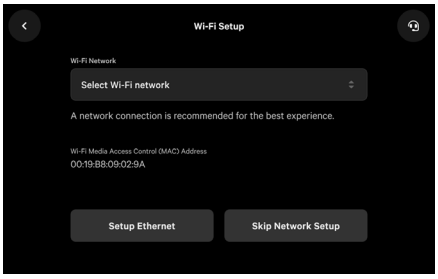
- 10x Genomics Account
  Onscreen instruction will guide account setup. Alternatively, visit cloud.10xgenomics.com to create an account beforehand.
- Instrument serial number and instrument ID
  Located onscreen during setup, and also on the label at the back of the instrument
- Network settings
- A computer with Internet access
- Internet connection to the instrument is highly recommended.
  Note that Wi-Fi configuration may not be available in your region. Ethernet is recommended for optimal performance.

Turn on the System

a. Power on the instrument using the power switch at the back of the unit.
If using Ethernet, connect the cable to the instrument Ethernet port.

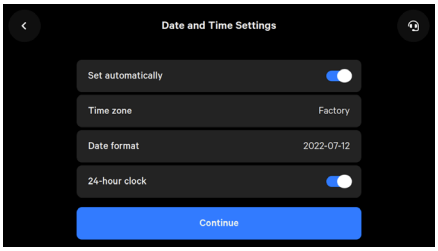
b. Follow onscreen instruction for Network Setup (via ethernet or Wi-Fi).





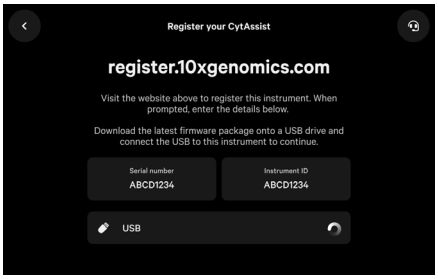
Step 2: First-Time Use *contd.*

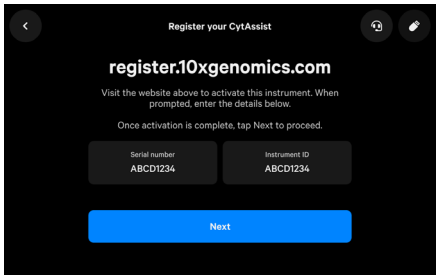
c. Set the Date and Time.



d. Register the instrument.

On a networked device, login to your 10x Genomics account at register.10xgenomics.com and register the instrument. Enter the serial number + instrument ID. Read and accept the legal agreement.





e. Update the firmware.

Internet connection: After registration, the instrument will automatically search for the latest firmware update. Confirm data transmission settings for automatic upload/download.

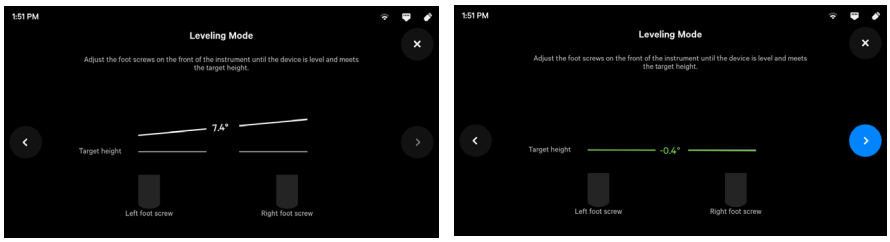
No Internet connection: Download the latest firmware update from your 10x Genomics account to the USB provided in the Instrument Accessory Kit. Insert USB into the back of the instrument.

Reference the Firmware Update Procedure section for more details.

Step 2: First-Time Use *contd.*

f. Proceed to Leveling Mode. To level the instrument, turn the adjustable feet at the front of the instrument. Adjust left-right tilt first by adjusting one foot first and adjust the front back tilt using both feet at the same time until the both bars turn green.

Turn feet clockwise for decreasing and counterclockwise for increasing height. The feet will come off the instrument if they are continued to be turned counterclockwise.



Once the instrument is leveled correctly, check the back of the instrument to confirm that the center back non-adjustable foot is resting on the table top while the two non-adjustable corner back bumpers are not touching the table top.

Back of a Leveled Instrument

See Troubleshooting section if an error occurs during the first-time setup protocol.

Step 3: Readiness Test

Prior to running the Readiness test, ensure that the Shipping Bolt has been removed.

The Readiness Test calibrates the imaging parameters and verifies that all systems are working optimally. No reagents are used (the Readiness Test is the only dry run the instrument utilizes). Wear gloves when performing the Readiness Test.

a. During first-time setup, follow onscreen instruction to launch the Readiness Test (~40 min).

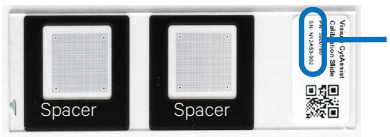
To initiate a Readiness Test at any other time, navigate through the menu to System > Alternate Modes > Readiness Test.

OR press the blue Run Start Button to initiate a run. Enter the Readiness Test serial number (located on the Visium CytAssist Calibration Slide label).

b. Locate the two blank slides and the Visium CytAssist Calibration Slide in the Instrument Accessory Kit. Inspect for any chips or cracks. *Calibration slide may be cleaned with lens paper or laboratory wipes followed by compressed air. Dirty slides may cause the readiness test to fail.*

c. Enter the serial number on the calibration slide (using a different serial number could result in poor calibration).

Calibration Slide

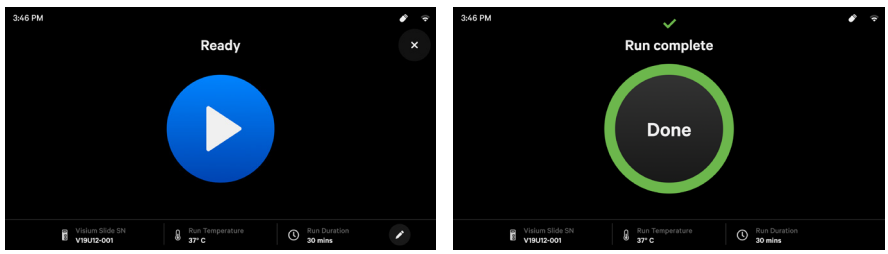


TIPS

d. Open the instrument lid.

e. Follow onscreen instruction and load the blank slides and calibration slide onto the instrument, as described in the Tips and Best Practices section. DO NOT load reagents to the calibration slide for the Readiness Test.

f. Press the blue play button to start the test.



g. A successful Readiness Test marks the end of setup. Follow onscreen instruction in the event of a failed or incomplete test.

Step 3: Readiness Test *contd.*

Clean Up After a Readiness Test

h. Upon test completion, open the lid and unload the blank slides and calibration slides. Calibration slide may be cleaned with lens paper or laboratory wipes followed by compressed air.

Calibration slide may be cleaned with lens paper or laboratory wipes followed by compressed air.

i. Store the blank slides and the calibration slide away from sunlight and extreme temperature, in the original packaging bag. Retain for future use.

j. Wipe down the Tissue Slide Stage and the Visium Slide Stage with 5-10 % bleach solution followed by 70% ethanol or 70% isopropanol. Use compressed air to dry & remove debris as needed. Clean glass windows with dry polyester swabs (such as Texwipe TX714A Cleanroom Swab, 0.5"L - EW-33677-62 from Cole-Parmer or equivalent) and compressed air. Use a bright light (e.g. flashlight) to inspect the glass windows for any smudges and remove them as described above.

Wipe the stages down with 5-10% bleach solution followed by 70% ethanol

k. Close the lid (clicking sound indicates closure; always store the instrument with the lid closed). Wipe down the base and lid of the instrument.

l. The instrument is now ready for use.

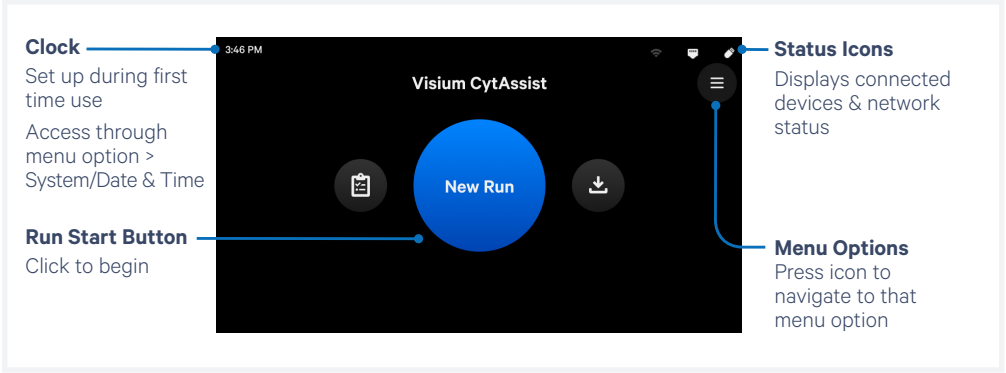
For optimal performance, Readiness Tests are recommended at the following times:

- During first-time use
- After any firmware update
- After moving the instrument

The instrument requires a **~20 min cool down** between runs.

Step 4: Main Operation

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



Main Operation Overview

This section is meant as a general overview of instrument operation and its placement within the steps of the Visium CytAssist Spatial Gene Expression assay. When performing the protocol, follow the steps listed in the appropriate assay kit user guide and use this section only as a reference.

Before proceeding with the appropriate Visium CytAssist Spatial Gene Expression assay:

Select two tissue slides containing sections of interest, and verify that the tissue section is located in an area that results in successful analyte transfer and imaging. Overlay the slides on the diagrams in the Visium CytAssist Quick Reference Cards – for Accessory Kit Instruction (CG000548), located in the Instrument Accessory Kit. *Refer to the Tips & Best Practices section for further guidance.*

If only one tissue on one slide will be loaded, use a blank slide for the second position on the Tissue Slide Stage.

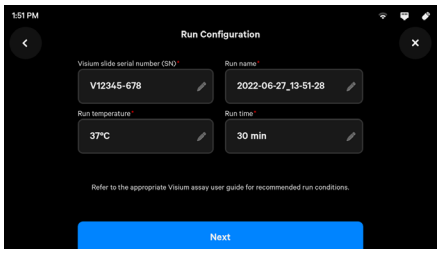
Follow the protocol in the appropriate assay kit user guide for preliminary steps and preparation prior to using the Visium CytAssist instrument.

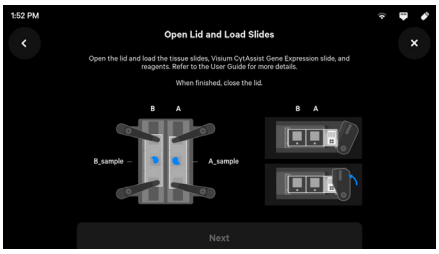
a. Gather two tissue slides, the appropriate Visium CytAssist Spatial Gene Expression slide and reagents as listed in the appropriate assay kit user guide.

Step 4: Main Operation *contd.*

b. Enter new run information, including:

- Visium CytAssist Slide serial number
- Custom run name, temperature and time (37 °C for 30 min is recommended for most applications)
  See the appropriate assay kit user guide for further guidance.
- Sample names; the 6.5 mm Capture Areas are designated **A1** and **D1**. The 11 mm Capture Areas are designated **A** and **B**.



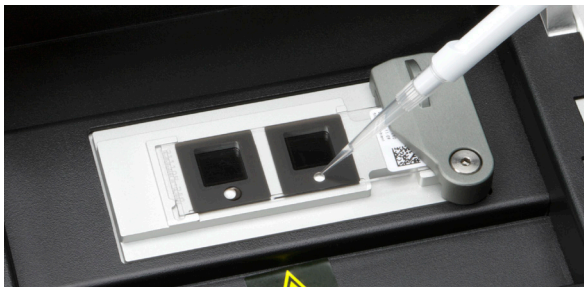


c. Open lid.

TIPS

d. Follow onscreen instruction and load the blank slides and calibration slide onto the instrument, as described in the Tips and Best Practices section.

e. Load reagents to the circular dispensing well on each spacer when instructed in the appropriate assay kit user guide (be mindful of time-sensitive reagents).



f. Close the lid (clicking sound indicates closure).

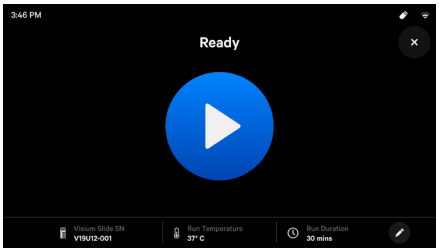
Pinch risk: Ensure no obstructions or fingers present when closing lid.

The home screen will now display a play symbol and run information along the bottom of the screen. (Use button in upper right corner of the screen to abort).

Step 4: Main Operation *contd.*

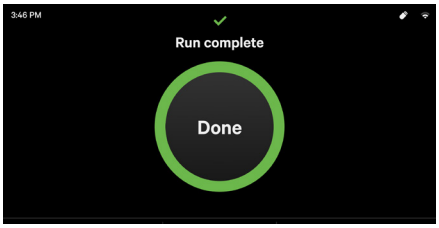
g. Press the play button to start the run.

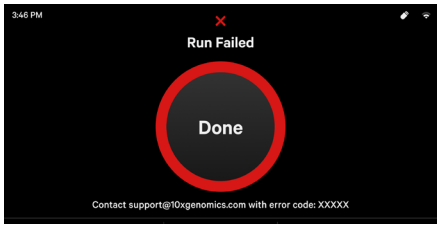
- Midrun progress bar will show the time remaining in the run.
- Yellow before a run begins indicates a user-recoverable error (see Troubleshooting).

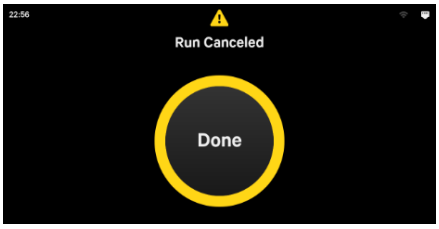


h. At the end of a run, the button will display "Done" and a "Run Info" tab at the bottom of the screen.

- Green indicates a successfully completed run.
- Red indicates a failed run/error (see Troubleshooting).
- Yellow indicates that the run has been canceled.







i. Click the "Done" button and open lid. DO NOT power off the instrument at this time, as it needs to process support data.

j. **IMMEDIATELY** remove the Visium CytAssist slide from the instrument, and proceed with the protocol workflow as specified in the appropriate assay kit user guide (save cleanup for later).

Step 4: Main Operation *contd.*

During the next stopping point in the workflow:

k. Clean the instrument after every run. *See* Maintenance *section for further guidance.*

l. Export the run data via USB. *See* Run Data *section for further guidance.*

m. View and verify the tissue samples were accurately aligned within the Visium CytAssist Slide fiducial frame. *See the relevant assay kit user guide for further guidance.*

The instrument requires a **~20 min cool down** between runs.

10x GENOMICS

Maintenance

System Maintenance

Clean Up After a Run

a. **After a Readiness Test or Training Run:** Open lid and store the blank slides and the calibration slide away from sunlight and extreme temperature, in the original packaging bag. Retain for future use. Proceed to step c.

After a Run: IMMEDIATELY open lid after a successfully completed run, remove Visium CytAssist Spatial Slide from the instrument, and proceed with the protocol workflow. During the next stopping point in the workflow, proceed to step b.

b. Dispose of tissue slides appropriately.

c. Wipe down the Tissue Slide Stage and the Visium Slide Stage with 5-10 % bleach solution followed by 70% ethanol or 70% isopropanol. Use compressed air to dry and remove debris as needed. Clean glass windows with dry polyester swabs (such as Texwipe TX714A Cleanroom Swab, 0.5"L - EW-33677-62 from Cole-Parmer or equivalent) and compressed air. Use a bright light (e.g. flashlight) to inspect the glass windows for any smudges and remove them as described above.

Wipe the stages down with 5-10 % bleach followed by 70% ethanol

d. Close the lid (clicking sound indicates closure). Wipe down the base and lid of the instrument.

See System Components section for interior and exterior images

Interior

The Visium Slide Stage of the Visium CytAssist has been designed to catch and contain drips and a small volume of liquid spills. Occasionally, use a soft lab towel to clean these areas with a mixture of mild detergent and distilled water. For deeper, more thorough cleaning, it is acceptable to use a 5-10% Bleach solution followed by a 70% ethanol or 70% isopropanol wash. Dry using compressed air.

Do not use acetone or other harsh solvents. Apply all standard safety practices when using cleaners, and dispose of any generated waste in a responsible manner.

System Maintenance *contd.*

Exterior

The exterior of the Visium CytAssist should always be kept clean and free of dust and debris that may affect its function and/or cooling efficiency. Generally, the exterior finish can be wiped down using a mixture of mild detergent and distilled water applied to a slightly dampened lab towel. As an added precaution it is recommended that the instrument be unplugged from the power source before beginning any thorough cleaning process.

Service

Electrical shock hazard. Do not open the Visium CytAssist in a manner not specified during standard operation. There are no user-serviceable parts inside. Refer all servicing to qualified 10x Genomics service personnel.

Servicing is required when the Visium CytAssist has been damaged in any way (e.g., a power entry module or plug is damaged, liquid was spilled into, or objects fell into the instrument, the instrument does not operate properly, or has been dropped).

Use only the power cord supplied with the Visium CytAssist. Do not replace it with a non-approved power cord as it may be inadequately rated to handle the electrical loads.

If replacing the externally accessible fuses in the power entry module becomes necessary, use only certified (EN60127 Sheet 5) 5 x 20 mm sized fuses rated T3.15AH, 250V Slow-Blow or equivalent.

When returning a Visium CytAssist for repair, take steps to ensure that the instrument has been decontaminated so as not to pose a hazard for 10x Genomics service personnel.

Environmental Requirements

It is the design intent of the Visium CytAssist that it be used in a typical indoor laboratory environment. The instrument's operating temperature is 18–28°C (64–82°F), humidity 80% Max (Non-Condensing) (see Product Specifications).

Moving & Shipping the Instrument

If the Visium CytAssist will be transported in a vehicle or shipped, it should be repackaged according to the instructions below. Run a new Readiness Test (see Readiness Test section) after relocating the instrument.

In the event of an instrument return to 10x Genomics, Support will guide the user through the correct documentation, in addition to the packaging procedure below.

Step 1: Shipping Mode

a. Launch Shipping Mode (for safe instrument transportation).
Menu Options > System > Alternate Modes > Shipping Mode

b. Once Shipping Mode is active, follow onscreen instruction to power down the instrument.

c. Turn off the instrument completely using the power switch on the back of the instrument.

d. Remove the power cord.

e. Decontaminate the instrument (see System Maintenance section).

Step 2: Packaging Instructions

a. Fully tighten the two adjustable feet at the front of the instrument by turning them clockwise.

b. Retrieve the Shipping Bolt from storage. Insert and tighten the Shipping Bolt at the back of the instrument (the Shipping Bolt holds the camera focus in a safe position during transportation).

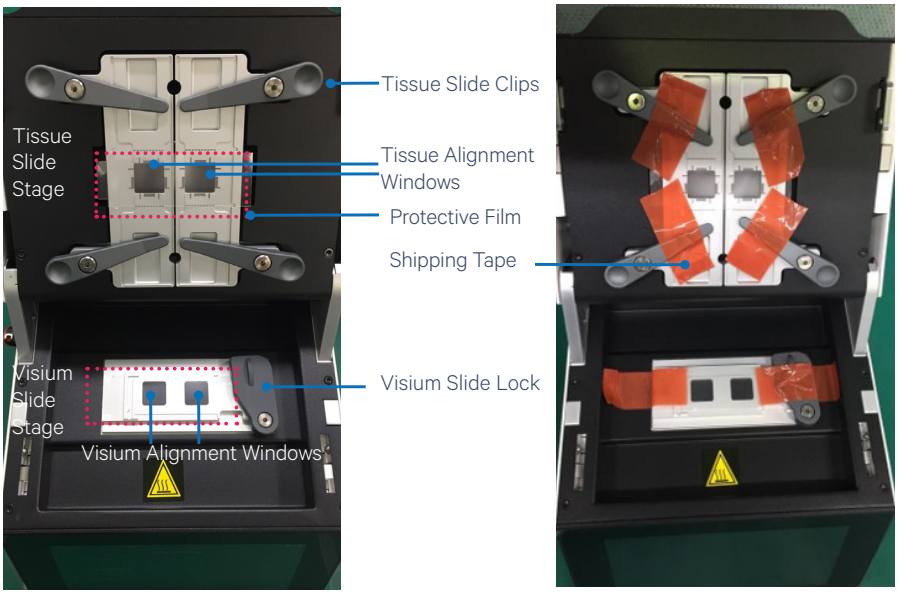
Moving & Shipping the Instrument
contd.

c. Open lid. Apply glass protective film or laboratory labeling tape to the Visium and Tissue Alignment Windows (placement indicated by red dashed line in the illustration below).

d. Move the Visium Slide Lock to the closed position and tape down the Visium Slide Lock and also the opposite end to secure the protective film.

e. Move the four Tissue Clips to their innermost positions and tape down. Ensure that the tape is placed over all four Tissue Slide Clips along with the overlap with all four corners of the protective film.

Apply glass protective films and then shipping tape



f. Close lid. Apply laboratory labeling tape to one side of the base, across the top of the lid, and down the other side of the base (prevents the lid from opening during transport).

Moving & Shipping the Instrument
contd.

g. Place the instrument within a plastic bag.

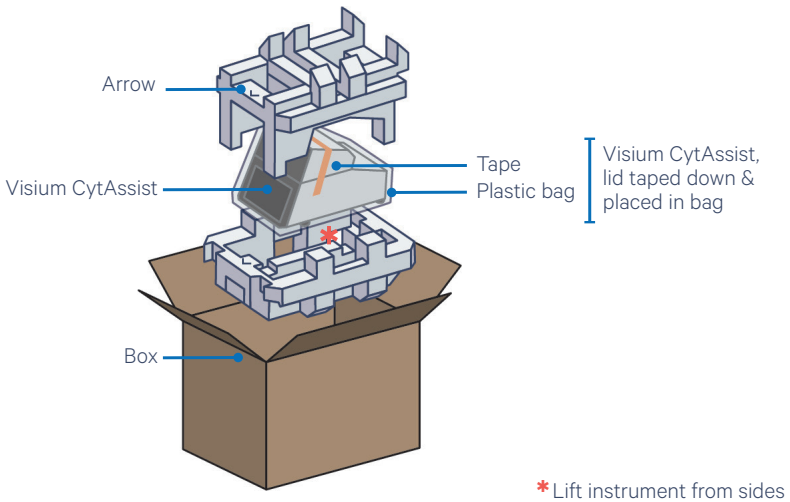
h. Place the foam packaging base within the box. The arrow on the foam indicates the front of the instrument. Insert the instrument, lifting from the sides. Place the foam packaging on top of the instrument.

i. Unless directed otherwise, do not include any parts of the Accessory Kit (which includes the power cord).

j. Coordinate with 10x Genomics Support for further return instructions (some required paperwork will be placed in the box). Place the required documents into the box.

k. Seal the box, and apply the shipping label.

l. The instrument is now ready for transportation.



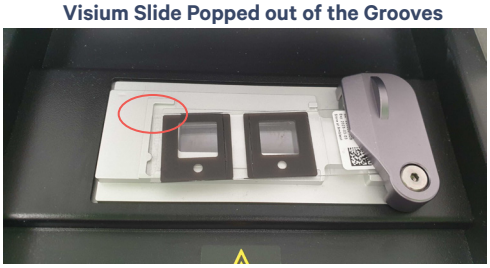
Troubleshooting

Troubleshooting

Issue

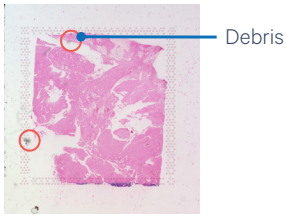
- Upon opening the lid after a successful run, **the Visium slide has popped out of the grooves** on the Visium Slide Stage. This does not impact assay performance or slide quality.

Visium Slide Popped out of the Grooves



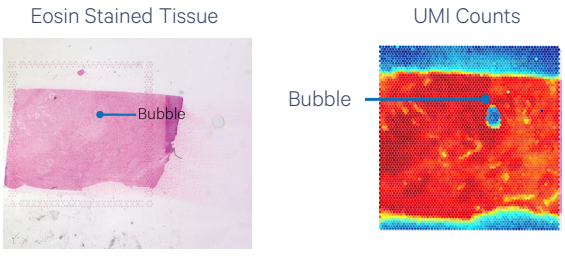
- **Debris is present** on the Tissue Slide Stage or Visium Slide stage: see System Maintenance section to clean the area.

Debris on the Slide Stage



- **Bubbles near tissue on tissue slide**: if bubbles are outside of the tissue, the risk of affecting assay performance is low. If bubbles are on top of the tissue, it may result in lack of usable reads in the tissue area with the bubble.
  Best practice: ensure reagent is prepared properly and correctly dispensed to the wells to avoid introducing bubbles.

Bubble on Tissue

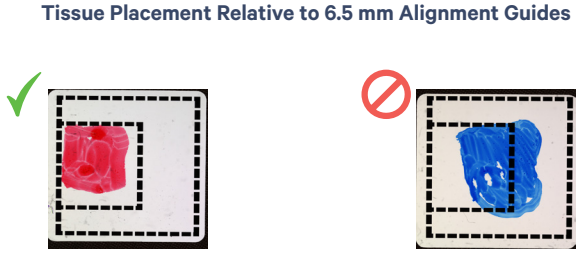


Some eosin may be washed off during a run, as shown on the right side of the image. This does not impact assay performance

Troubleshooting
contd.

- **Instrument is hot**: If the Visium CytAssist overheats during an experiment run, the run will fail. A warning message will appear instruting users to carefully remove slides and contact support@10xgenomics.com

- **Poor tissue alignment on Visium CytAssist Slide**: Refer to the Tips & Best Practices section to verify tissue placement on a tissue slide. Practice using the Visium CytAssist Training Kit and User Guide (CG000549).



- **Readiness Test Fails:** Refer to the Maintenance section and clean the instrument as described. Wipe down the Tissue Slide Stage and the Visium Slide Stage with 5-10 % bleach solution followed by 70% ethanol or 70% isopropanol. Use compressed air to dry and remove debris as needed. Clean glass windows with dry polyester swabs (such as Texwipe TX714A Cleanroom Swab, 0.5”L - EW-33677-62 from Cole-Parmer or equivalent) and compressed air. Use a bright light (e.g. flashlight) to inspect the glass windows for any smudges and remove them as described above.
If the problem persists, contact support@10xgenomics.com.

Error Screens

The touchscreen will guide the user through recoverable errors. If the error continues, or if the instrument has seen critical or intermediate errors, email support@10xgenomics.com with the error code displayed.

Support will request log files for troubleshooting. Upload pertinent logs to 10x Genomics by navigating to the Logs menu option on screen. (See Run Data section).

There are two types of errors:

Critical Errors – When the instrument has seen a critical error, the run will immediately abort. Do not proceed with any further runs. Contact support@10xgenomics.com with the error code.

- System Error
- Run Error
- Canceled

User Recoverable Errors – Follow error handling instructions through the touchscreen and continue the run.

- Slide Error
- Network Error
- Update Error