10x GENOMICS

User Guide | CG000584 | Rev C

Xenium Analyzer

For use with:

Xenium Analyzer with 12-Month Warranty, PN-1000481
(Includes Xenium Instrument Bundle, PN-1000569 - Xenium Analyzer, Analysis Computer, Instrument Accessory Kits)

Notices

Document Number

CG000584 | Rev C

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

CG000584 | Rev C

Title

Xenium Analyzer User Guide

Revision

Rev B to Rev C

Revision Date

September 2023

Specific Changes

- Updated to remove powering off language in Safety/Compliance section (page 13)
- Updated information including storage and handling conditions for Decoding Reagent Modules A and B (pages 24, 32, 45, 47-49)
- Added more guidance on deselecting FOVs (page 31)
- Added section called “Software Update” to reflect new guidance on customer driven software updates following v1.6 release (pages 41-43)
- Added greater detailed guidance on Sample Scanning (pages 65-67)
- Added note in Region Selection section that only alphanumeric characters are allowed in region selection name (page 68)
- Updated guidance on Data Export (page 79)
- Added subsection describing cassette carrier cleaning in the Maintenance section (page 81)

General Changes

- Updated for general minor consistency of language, terms, images, and format throughout

Table of Contents

Introduction

Introduction

Xenium In Situ is the next-level in situ solution for subcellular profiling of hundreds of RNA targets. Xenium Analyzer combined with our curated and customizable panels, powerful visualization software, and easy-to-follow workflow is a powerful in situ profiling platform, revealing new insights into cellular structure and function.

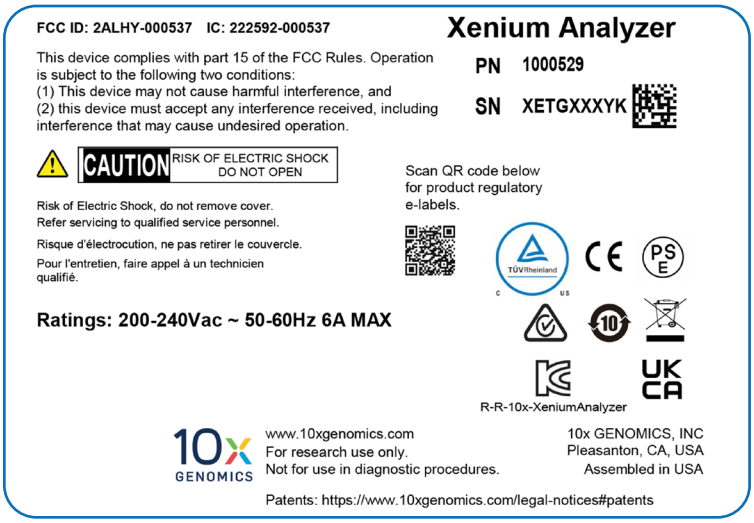
Xenium In Situ provides highly sensitive, targeted gene expression information at subcellular resolution for hundreds of RNA targets, in fresh frozen (FF) and formalin fixed & paraffin embedded (FFPE) tissue. Primary analysis to decode image data to transcripts and secondary analysis to segment cells and assign transcripts is performed automatically, directly on-instrument. Using Xenium Explorer, view and explore this on-instrument output to see where cells belonging to clustering results are localized, annotate clusters based on expression patterns, overlay individual transcripts at sub-cellular resolution with morphology images and cell segmentation boundaries, and compare expression between selected regions. The key attributes of Xenium Analyzer are highlighted below.

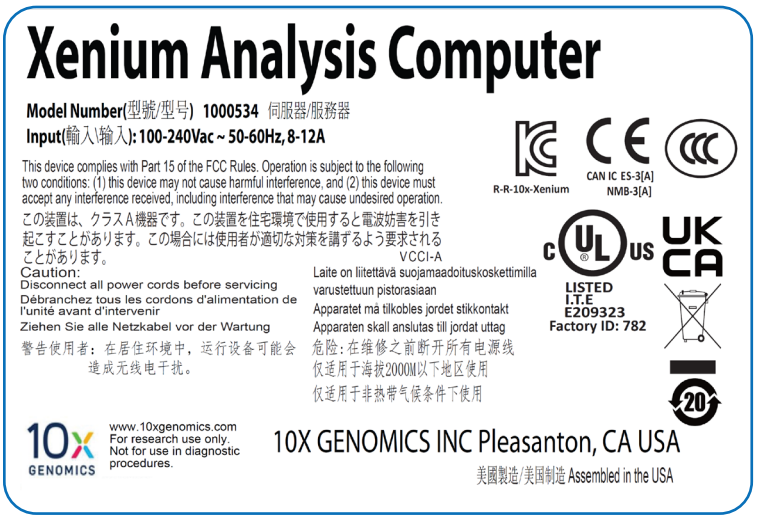
Highlights

- **Sample input flexibility**: Compatible with fresh frozen (FF) and formalin fixed & paraffin embedded (FFPE) tissues.
- **Curated targeted gene panels with custom capabilities**: Choose from pre-designed panels or customize a pre-designed panel.
- **Increased speed and throughput**: Large sample area (12 x 24 mm) on the Xenium Slide allows for larger tissues or multiple tissues to be included in a single run, increasing efficiency and saving time.
- **Intuitive instrument design and interface:** Get started quickly with the easy to use instrument design and interface.
- **Robust, flexible platform**: Automated in situ platform that performs successive rounds of fluorescent probe hybridization, imaging, and probe removal to generate an optical signature for each transcript.
- **Onboard analysis:** Image processing, decoding, and secondary analysis are performed real time on-instrument, resulting in a cell-feature matrix and initial clustering results.
- **Data visualization:** Data can be transferred off the instrument and visualized with the free Xenium Explorer desktop software. Xenium Explorer allows for immediate interactivity with on-instrument output, including overlays of transcripts at subcellular resolution, morphology images, segmentation results, and cluster localization. Xenium data is in an open file format, making it compatible with a wide variety of open source software tools.

Product Identification

The product label is located at the back panel of the instrument. Images of the labels below are for reference only.





Instrument Specifications

Parameter	Xenium Analyzer Specifications		
Weight			
Xenium Analyzer	~550 lb/249.5 kg		*Total weight of system: ~1,107 lb (502.1 kg)*
Xenium Analysis Computer	~57 lb/25.8 kg		
Vibration Isolation Table	~500 lb/226.8 kg		
Dimensions	**L**	**W**	**H**
Xenium Analyzer	52.5"/133.3 cm	27"/68.5 cm	31"/78.7 cm *59"/149.8 cm - door open*
Xenium Analysis Computer	7"/17.8 cm	26.5"/67.3 cm	18"/45.7 cm
Vibration Isolation Table	53.2"/135.0 cm	29.9"/76.0 cm	29.6"/75.2 cm
UPS *(APC SRT3000XLT or similar; not provided by 10x Genomics)*	3.4"/8.5 cm	25"/63.5 cm	17"/43.2 cm
Xenium Analyzer Electrical Specifications	200-240 VAC, 50-60 Hz, 6 A*		
Pollution Degree	2 (Indoor Use Only)		
Operating Temperature	19-25°C (66-77°F) *Use in a typical indoor laboratory environment. Extreme temperature conditions will affect the sensitive reagents used with the instrument.*		
Humidity	30-80% Relative Humidity, non-condensing		
Altitude	Altitude up to 2,000 m (1.2 mile) above sea level		
Environmental Vibration Guidelines	ISO Office (or better) during idle ISO Operating Theater (or better) during run *No bumps or shocks adjacent to or on the Vibration Isolation Table during a run*		
Heat Output	~2,000 W (6,820 BTU/h) *Combined output from the Xenium Analyzer & the Xenium Analysis Computer*		
Power Cable Length	~1.83-3 m (~6-9.8 ft) *Cables will be in accordance with regional specifications*		
Xenium Analysis Computer Specifications	RAM: 1TB DDR4-3200 ECC RDIMM Storage Capacity: 8 TB NVMe (stores data from more than 50 runs**) Operating System: Ubuntu 22.04 LTS (non-configurable)		
Xenium Analysis Computer Electrical Specifications	200-240 VAC, 50-60 Hz, 6 A*		

**Electrical requirements dependent on region/country*

***Assuming data is acquired across the full imaging area of two Xenium slides per run for hundreds of RNA targets. To reduce system load and to avoid any possibility of losing run data, exporting the output data after each instrument run is highly recommended. For guidance on exporting data, follow the Xenium Analyzer user interface and refer to the Data Output chapter in this document for additional details.*

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Safety & Compliance Information

Xenium Analyzer
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.
	The Caution, Hot Surface symbol indicates the possibility of touchable surface that may exceed 105°C.

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.*

Warning: The door is capable of moving an object that is in its opening path. If an object is in the path, the object could fall and create a hazard.

Pinch risk: Ensure no obstructions or fingers present near closing trays. Once the system is floating, keep fingers away from the area between the support plate and the top of the isolators. Any object between these points may be caught if the load or air supply changes.

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

Heavy Load: 1,107 lb (502.1 kg). Contact 10x Genomics Service Personnel for Lifting and Installation.

Xenium Analyzer
Regulatory

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

Certification	Standards
	TUV Certification only for Xenium Analyzer 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 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.
	Complies to Japan's Ministry of Economy, Trade and Industry (METI) Electrical Appliance and Material Safety Law (DENAN). This is a Class A product based on the standard of the Voluntary Control Council for Interference (VCCI). If this equipment is used in a domestic environment, radio interference may occur, in which case the user may be required to take corrective actions. これは電波障害自主規制協議会 (VCCI) の基準に基づくクラス A 製品です。 この装置を家庭環境で使用すると、無線干渉が発生する可能性があり、その場合、ユーザーは是正措置を講じる必要があります。 VCCI-A

Xenium Analysis Computer
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.

Warning: Read the installation instructions before connecting the system to the power source.

Warning: Only trained and qualified personnel should be allowed to install, replace, or service this equipment.

Warning: Installation of the equipment must comply with local and national electrical codes.

Warning: Keep fingers, screwdrivers, and other objects away from the openings in the fan assembly's housing.

Warning: When installing the product, use the provided or designated connection cables, power cables, and AC adaptors. Using any other cables and adaptors could cause a malfunction or a fire.

Xenium Analysis Computer
Regulatory

The Xenium Analysis Computer has been designed, tested, and certified to be in compliance with the following standards:

Certification	Standards
	UL Certification only for Xenium Analysis Computer UL 62368-1: 2019 and CAN/CSA-C22.2 NO. 62368-1:12 with a cULus mark to indicate that the product has been tested and certified to Canadian and US standards by UL and can be legally installed in those countries.
	IEC 62368-1: Audio/video, information and communication technology equipment - Part 1: Safety requirements.
	EN 55032:2015+A11:2020 (Class A) - Electromagnetic compatibility of multimedia equipment - Emission Requirements EN 55035:2017+A11:2020 - Electromagnetic compatibility of multimedia equipment - Immunity 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 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 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.
	China CCC: GB 17625.1-2012;GB 4943.1-2011;GB/T 9254.1-2021(Class A).
	Complies to Japan's Ministry of Economy, Trade and Industry (METI) Electrical Appliance and Material Safety Law (DENAN). This is a Class A product based on the standard of the Voluntary Control Council for Interference (VCCI). If this equipment is used in a domestic environment, radio interference may occur, in which case the user may be required to take corrective actions. これは電波障害自主規制協議会 (VCCI) の基準に基づくクラス A 製品です。 この装置を家庭環境で使用すると、無線干渉が発生する可能性があり、その場合、ユーザーは是正措置を講じる必要があります。 VCCI-A

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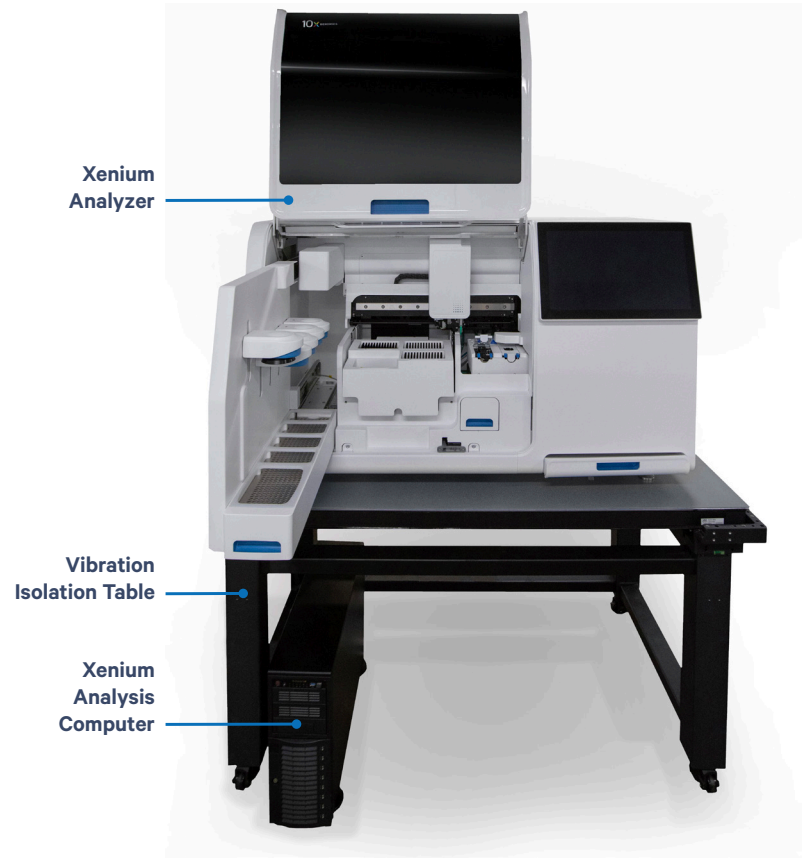
System Components

Instrument Installation

10x Genomics will provide complete installation services necessary for Xenium Analyzer, Vibration Isolation Table, and Xenium Analysis Computer.

Full Instrument Setup



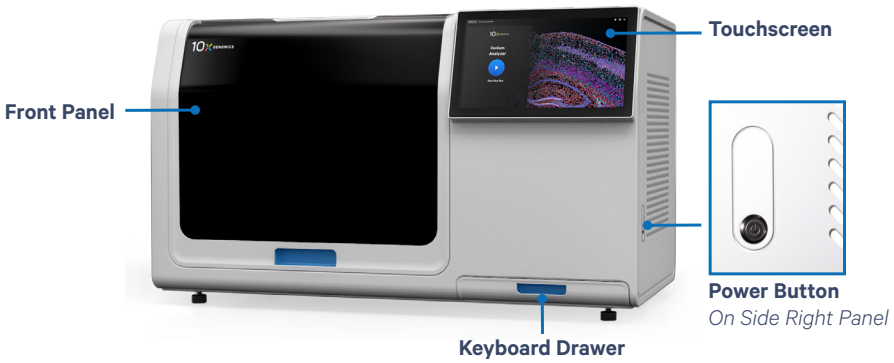
System Components - *Xenium Analysis Computer*

Front

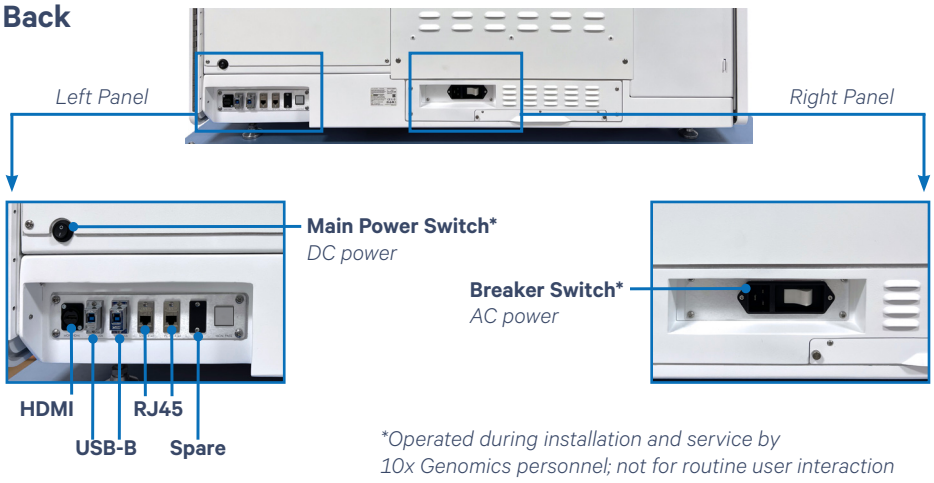
**Operated during installation and service by 10x Genomics personnel; not for routine user interaction*

System Components - *Xenium Analyzer*

Front



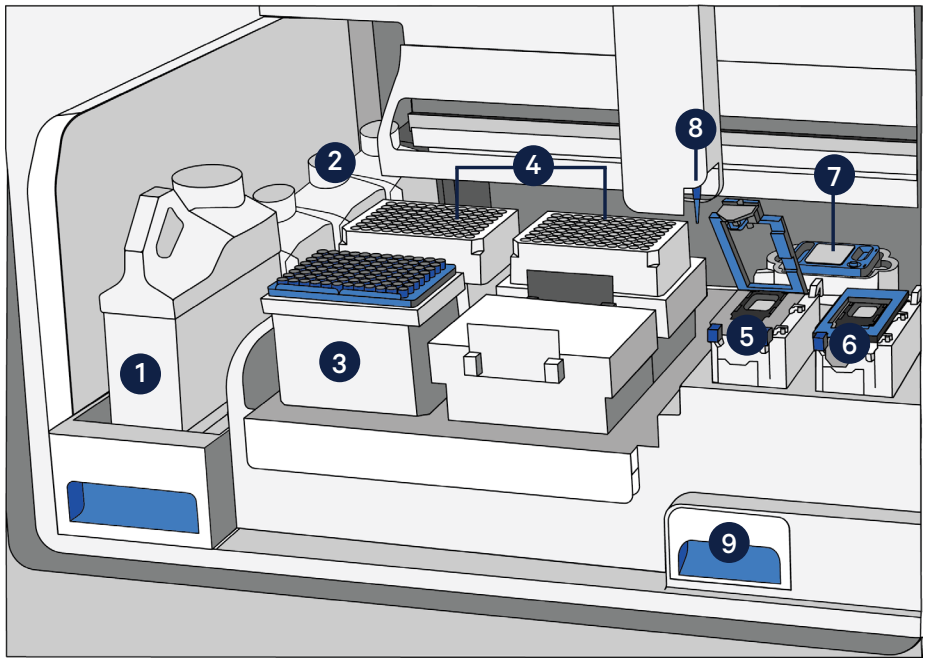
Back



*Operated during installation and service by 10x Genomics personnel; not for routine user interaction

Inside (Hardware)

Deck Layout - *Xenium Analyzer*



Location		Part
Bottle Carrier	1	Waste Bottle
	2	Reagent Bottles *(4 total)*
Plate Deck	3	Pipette Tip Rack
	4	Reagent Plates *(2 total)*
Cassette Carrier	5	Left Cassette *(lid open)*
	6	Right Cassette *(lid closed)*
	7	Objective Wetting Consumable
	8	Extraction Tip
	9	Waste Tip Drawer *(Waste tip tray inside)*

Hardware Components - *Xenium Analyzer*

The Xenium Analyzer includes the following hardware components designed for seamless workflow execution. Refer to the System Components and Deck Layout sections for the specific location of each hardware component.

Touchscreen

The touchscreen is located on the right side of the instrument. Interaction with the software user interface is performed here.

Avoid using the touchscreen and keyboard during instrument runs; if needed, use the touchscreen instead of the keyboard.

Keyboard Drawer

The wireless keyboard drawer is located on the bottom right corner of the instrument, underneath the touchscreen. A keyboard with trackpad is provided to aid in navigating the user interface.

Bottle Carrier

Within the instrument deck, the bottle carrier is located at the far left. It can be pulled out using the handle and the Waste Bottle and reagent bottles are housed in the carrier.

Waste Tip Drawer

The waste tip drawer is located toward the bottom right of the instrument deck. Pulling the handle to open will reveal the Waste Tip Tray where all solid waste (i.e. tips) is deposited during a run.

Plate Deck

The plate deck is in the center of the instrument deck. Pipette tips and reagent plates are housed here.

Cassette Carrier

Two cassette carriers sit to the right of the plate deck. Tabs in the front enable opening of the carriers to allow for slide cassettes to be loaded.

Carrier lid must be fully opened prior to loading the slide cassette. If not fully open and/or the slide is not sitting correctly, closing the carrier lid will result in crushing/breaking of the assembled slide.

The cassette carrier surface (center black area) will be hot during a run.

Caution: Hot surface

Accessories & Consumables

The Xenium Analyzer uses the following accessories and consumables required for operation. Unless otherwise noted, each consumable is good for **one run and must be replaced** before the start of each run. Refer to the Deck Layout section for specific locations of each item.

Not all accessories and consumables are shipped with the instrument. Refer to the Accessory Kits and Reagent Kits and Consumables sections for complete details.

Objective Wetting Consumable

The Objective Wetting Consumable sits behind the slide cassettes/cassette carrier. It has four parts: the reagent priming reservoir, the objective drying pad, the objective wash reservoir, and the extract orifice.

Waste Bottle *(Reusable)*

The liquid Waste Bottle sits in the front position of the bottle carrier. It serves as a receptacle for all liquid waste that is generated during a Xenium run. The Waste Bottle is reusable and should be emptied after each run when prompted and returned back to position before starting the next run.

Follow institutional or local guidelines for proper liquid waste disposal

Accessories & Consumables *contd.*

Waste Tip Tray *(Reusable)*

The Waste Tip Tray sits inside the waste tip drawer. Solid waste (i.e. tips) generated during each run is stored in the waste tip container. It can be reused between runs, but must be emptied after each run.

Follow institutional or local guidelines for proper solid waste disposal

Reagent Bottles (x4)

Four reagent bottles are provided and sit behind the Waste Bottle in the bottle carrier. Each bottle will be filled with a specified buffer for use in various steps in the Xenium Analyzer workflow. Place the bottles in the correct order, as specified on the touchscreen. Bottles are color coded to help with correct loading. Bottle position is noted on the instrument. Reagent bottles are for **single use only**, discard after each run.

Pipette Tip Rack

Single stack of 1 ml pipette tips sit in the front left area of the plate deck and labeled with a T on the front. Tips are used to facilitate the movement of liquids during an experiment. **Single use only**, discard after each run.

Reagent Plates (x2)

Two reagent plates (Plate A and B) are provided and sit within the plate deck behind the Pipette Tip Rack. Each reagent plate is specific to its location and contains unique reagents required to properly carry out a successful run on the Xenium Analyzer.

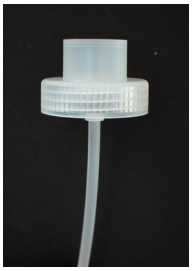
Plates require specific handling and preparation prior to loading and are described in detail in Reagent Plate Preparation section. All plates are **single use only** and must be discarded after each run. The foil seal on the plates should be intact when loaded on the instrument. DO NOT use plates if foil seal is punctured.

Accessories and Consumables *contd.*

Extraction Tip

The Extraction Tip is used to facilitate liquid waste transfer from the Objective Wetting Consumable to the Waste Bottle container and to aspirate liquid from the loaded cassette. The Extraction Tip is a **single use item** and must be discarded after each run.

Xenium Buffer Cap



Reagent bottles must be capped using the Xenium Buffer Cap (includes an integrated straw) prior to loading onto the instrument. These special caps allow for bottles to be locked into place in the bottle carrier. Four are provided (one per reagent bottle); all are **single use only** and must be discarded after each run.

Accessory Kits

Xenium Instrument Bundle, PN-1000569

Includes Xenium Analyzer and Xenium Analysis Computer (PN-1000529)*
Instrument Accessory Kit Module A & Module B

**Xenium Analysis Computer only PN-1000534*

Xenium Instrument Accessory Kit Module A, PN-1000530
shipped with instrument

Item	#	Part Number
Waste Bottle	1	3000955
Xenium Waste Tip Tray	1	3000957
Xenium Thermocycler Adaptor*	1	3000954

**The Xenium Thermocycler Adaptor is required for sample preparation performed prior to instrument loading (Documents CG000578, CG000580, CG000581, CG000582).*

Xenium Instrument Accessory Kit Module B, PN-1000582
shipped with instrument

Item	#	Part Number
Coolant Bottles	2	3001331
Ethernet Cable, 8 ft.	2	3001335
Ethernet Cable, 20 ft.	1	3001611
HDMI Cable	1	3001337
USB Cable, 3.0 A Male to B Male	1	3001336
Foot Mounting Brackets	4	3001765
Foot Mounting Screws**	10	3001766

***Eight screws are required for Xenium Instrument. Two additional screws are provided.*

Region-specific Xenium Power Cable Kit will be shipped along with the Xenium Instrument Accessory Kit Module A.

Reagent Kits & Consumables

Refer to SDS for instructions on proper handling and disposal of volatile and hazardous chemicals.

The following reagent kits and consumables are needed for loading a Xenium Analyzer run. Additionally, some kit components are required for steps prior to instrument operation.

It is recommended to confirm that all reagents and kits are received prior to starting an experiment.

Xenium Decoding Consumables (1 run, 2 slides) PN-1000487

Items *(store at room temperature)*	#	Part Number
Xenium Cassette Kit* (2 cassettes + 16 lids)	1	1000566
Extraction Tip	1	2000757
Pipette Tips	1	3000866
Xenium Buffer Cap	4	3000949
Xenium Objective Wetting Consumable	1	2000749
1 Deionized Water (bottle)	1	3001198
2 Xenium Sample Wash Buffer A (bottle)	1	3001199
3 Xenium Sample Wash Buffer B (bottle)	1	3001200
4 Xenium Probe Removal Buffer (bottle)	1	3001201

**Required for sample preparation, which is performed prior to loading the instrument (Documents CG000580, CG000581).*

Xenium Decoding Reagents (1 run, 2 slides) PN-1000461

Items	#	Part Number
Xenium Decoding Reagent Module A (store at 4°C)	1	1000624
Xenium Decoding Reagent Module B (store at -20°C)	1	1000625

Decoding Reagent Modules A and B require different storage conditions. Refer to packaging for proper storage instructions upon receipt. Failure to comply with storage instructions will render reagents unusable.

Gene Panels

Prior to executing the Xenium In Situ Gene Expression workflow, ensure that a compatible gene panel has been selected. 10x Genomics provides the following types of probe panels: pre-designed, add-on custom, and standalone custom. Add-on custom panels are used to supplement pre-designed panels. Standalone custom probe panels are used alone and do not require pre-designed panels.

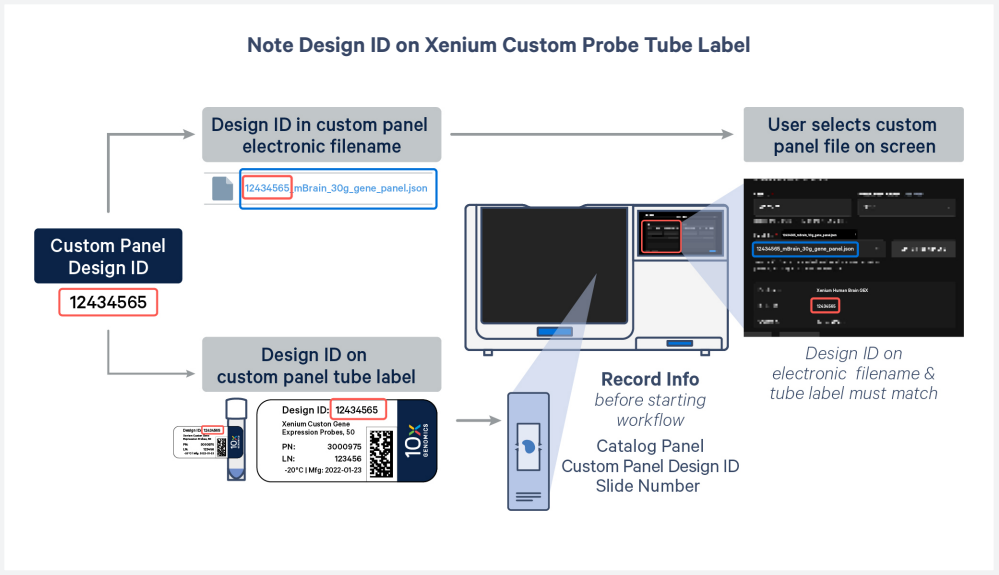
10x Genomics Pre-designed Gene Panels

Visit the 10x Genomics Support website for the most current information regarding all the available pre-designed panels.

Compatible Custom Gene Panels

Contact your 10x Genomics Sales Executive for information about designing custom gene panels that are compatible with pre-designed panels or standalone custom gene panels. If you do not know your Sales Executive, contact customerservice@10xgenomics.com. The lead time for acquiring custom panels is ~6 weeks (~3 week for gene selection/design, 3 weeks for manufacturing) from start of custom panel design to receipt of probes. Visit the 10x Genomics website for additional information.

If using a custom panel, note the Design ID on the label of the tube containing the panel. This Design ID on the tube label should match with the custom gene panel electronic filename that is selected on the touchscreen during instrument run (see Initialize Instrument section).



Additional Kits, Reagents & Equipment

The listed items have been tested by 10x and perform optimally with the assay. Substituting materials may adversely affect system performance. For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers.

For Reagent Bottle Buffer Preparation				
	Item	**Description**	**Vendor**	**Part Number**
☐	Nuclease-free Water	Nuclease-free Water (not DEPC-treated) Nuclease-free MilliQ water (Biopak® Polisher) *(select one based on availability)*	Thermo Fisher Scientific Millipore Sigma	AM9932/ AM9937 CDUFBI0A1
☐	PBS-T	Phosphate Buffered Saline with 0.05% Tween 20, pH 7.4 Phosphate Buffered Saline with 0.05% Tween 20, pH 7.4 *(select one based on availability)*	Millipore Sigma Millipore Sigma	P3563-10PAK PPB005-20PAK
☐	PBS *Alternate for making PBS-T*	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free	Thermo Fisher Scientific	AM9624
☐	Tween 20	Tween 20 Surfact-Amps Detergent Solution (10% solution) *(use one ampule per use)* 10% Tween 20	Thermo Fisher Scientific Bio-Rad	28320 1662404
☐	100% DMSO	Dimethyl sulfoxide (molecular biology grade) Dimethyl sulfoxide (molecular biology grade) Dimethyl sulfoxide (molecular biology grade) Dimethyl sulfoxide (molecular biology grade) Dimethyl sulfoxide, Fisher BioReagents (>99.7%) Dimethyl sulfoxide (for molecular biology, 99.5+%) *(select one based on availability)*	Millipore Sigma Millipore Sigma Millipore Sigma Millipore Sigma Fisher Scientific Fuji Film	41639-100 ML 41639-500 ML D8418-250ML D8418-1L BP231-1 043-29355 500 ml
☐	KCl	Potassium Chloride (KCl, sterile), 500 ml Potassium Chloride (KCl, sterile), 1L KCl (2 M), RNase-free *(conc. in working solution will be 50 mM; select one based on availability)*	Teknova Teknova Invitrogen	P0330 P0335 AM9640G
Additional Materials				
☐	Centrifuge with plate rotor	Allegra X-14 Series Benchtop Centrifuge 120 V *Or equivalent; fits deep-well 96 well plates (~2 ml vol.)*	Beckman Coulter Coulter	-
☐	Serological Pipettes	10 ml, 25 ml, 50 ml, 100 ml		
☐	Serological Pipette Controller	Compatible with 10, 25, 50 & 100 ml serological pipettes		
☐	Graduated Cylinders	100 ml and other volumes as needed		

Contd.

Additional Kits, Reagents & Equipment *contd.*

The listed items have been tested by 10x and perform optimally with the assay. Substituting materials may adversely affect system performance. For items with multiple options listed, choose option based on availability and preference. Refer to the manufacturer's website for regional part numbers.

Additional Materials				
☐	Pipette Tips	Tips LTS 1ML Filter RT-L1000FLR *Or equivalent*	Rainin	30389213
☐	Pipettes	Pipet-Lite LTS Pipette L-1000XLS+ *Or equivalent*	Rainin	17014382
☐	Glass Bottles with Cap	Pyrex Reusable Media Storage Bottles (500 ml and 1 l) *Or equivalent*		
☐	Compressed Canned Air for cleaning			
☐	Lens-cleaning Paper or Lint-free Laboratory Wipes *High-Tech Conversions ULTIMATE 9 Quilted 2-Ply Polyester Wipes from Fisher Scientific or equivalent*			
☐	70% Isopropanol			
☐	Laboratory Balance			
☐	Ultrapure water	Ultrapure/Milli-Q water, *from Milli-Q Integral Ultrapure Water System or equivalent*		

This list may not include some standard laboratory equipment.

Software Overview

On-Instrument Pipeline Overview

The Xenium Analyzer includes an on-instrument analysis pipeline. The Xenium Analyzer captures vertical stacks of images at every cycle (of fluorescent probe hybridization, imaging, and probe removal) and in every channel for multiple fields of view, which need to be processed, corrected and stitched to build a single seamless image representing the tissue section. Next, the pipeline detects puncta in every cycle and every image in order to observe all potential mRNA. These puncta are decoded into gene IDs, and each decoded transcript is assigned a quality score. In order to define cell boundaries and thus assign transcripts to cells, cell segmentation is performed using DAPI images (derived from Xenium Nuclei Buffer stained samples) and a neural network. Finally, the pipeline outputs a bundle of data files (see Data Output chapter) that can be exported for further downstream analysis.

Xenium Explorer Overview

The Xenium Explorer software can be installed on Mac or Windows computers for off-instrument downstream analysis and visualization. Xenium Explorer is an easy-to-use tool that enables users to zoom in and out of regions of interest, map gene expression data and cell segmentation boundaries, and assess cluster assignments to known tissue types as layers on top of DAPI-stained microscopy images. Users can check data quality and export or share data to inform downstream analyses.

Visit the 10x Genomics Support website for additional information.

Tips and Best Practices

TIPS

Icons

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

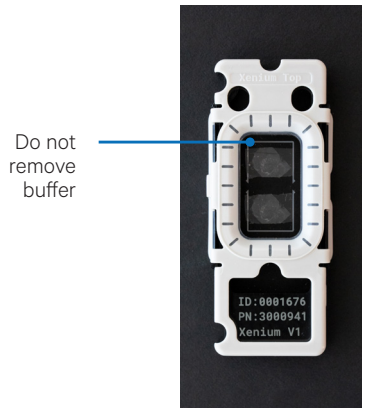
Troubleshooting section includes additional guidance

Handling Xenium Slide with Tissue Section

Ensure that the Xenium Slide with the tissue sections (processed and stored as per the off-instrument workflow described in the Xenium In Situ Gene Expression Probe Hybridization, Ligation & Amplification CG000582) is retrieved from storage just prior to loading the instrument.

- The Xenium Slide should continue to be in the Xenium Cassette with the lid on until prompted to remove. The lid may be saved for using later if storing the cassette assembly post-run.
- PBS-T (storage buffer) should not be removed to prevent tissue sections from drying.

Stored in PBS-T

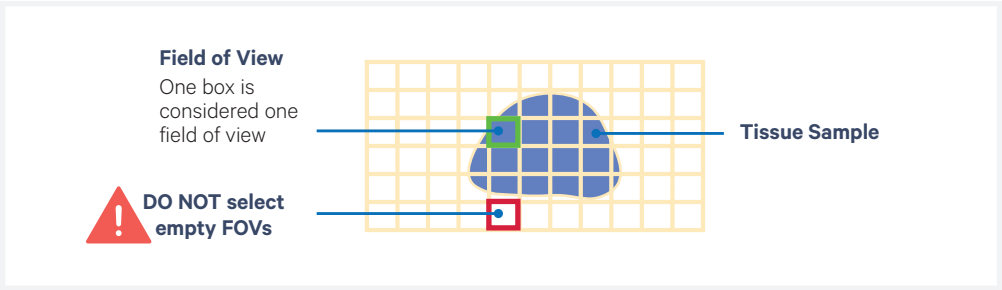


Region Selection Guidelines

During sample scanning, the user must define the regions of interest to scan prior to starting the instrument. Following guidelines on how to select the regions will help with more efficient scanning time and data analysis post instrument run.

Key considerations with selecting regions

- The unit of selection is called a field of view (FOV). One FOV corresponds to one box in the grid.



- At least one FOV must be defined as a region. At least one region must be defined per slide. Regions do not need to be contiguous.

Each FOV can only be assigned to one region and cannot be selected twice. DO NOT select empty FOVs. Selecting empty FOVs will yield stitching errors.

- **For slides with multiple tissue sections:**
  - Select each tissue section as a separate region
  - Exclude overlapping regions. If the overlapping region is assigned to one tissue, the overlapping area can be imaged but the data will be unusable.
  - For slides that contain > 8 sections, consolidating to one region is recommended as using high number of regions will increase the run time.

Reagent Plate and Buffer Preparation

Storing and Thawing Reagent Plates

Decoding Reagent Modules A and B are packaged in mylar bags for protection. All storage and thawing must be done in the mylar bag. Only when ready to use, open the mylar bag and remove the foil sealed plate prior to preparation for loading.

Decoding Reagent Module A is stored at 4ºC upon receipt and is ready to use on day of instrument. No thawing or equilibration necessary.

Decoding Reagent Module B is stored at **-20ºC** upon receipt and must be thawed at **4ºC** for **16-72 h** prior to handling and loading onto the instrument. For same day use, thaw plate at **37ºC water bath** for **2.5 h** in mylar packaging. Factor in the thawing step when planning an experiment.

Preparing Reagent Buffers

Reagent buffers must be prepared prior to filling the reagent buffer bottles and loading them on the instrument. Detailed instructions on how to prepare buffers are provided in Reagent Preparation section.

Cleaning Slides and Cassette Carriers

Cleaning the bottom of the Xenium slide and the cassette carrier prior to loading the assembled cassette is critical for a successful Xenium run. Any fingerprints, lint, or liquid may interfere with image acquisition that may result in a failed run or incomplete or unreliable data generation.

After a completed run, clean the carriers after unloading the slide, especially if liquid has leaked during the run to prevent liquid from drying onto the surface of the carrier.

a. Spray 70% isopropanol onto a lint-free laboratory wipe and clean the surface of the carrier, paying attention to the raised areas that come into contact with the slide. Let evaporate.

Cleaning Cassette Carrier

i. Optional: Spray 70% isopropanol on a cotton swab and use to clean off crevices if necessary.

b. Use compressed air to remove any remaining lint paying close attention to raised areas. Confirm surface is dry.

c. Check assembled cassette to ensure the seals are not leaking liquid by blotting bottom with lint-free laboratory wipe.

d. Clean the bottom surface of the slide with 70% isopropanol using a lint-free laboratory wipe without spilling the storage buffer. Confirm the bottom of the slide bottom is clean and dry.

A dry, clean, lint-free surface on both the slide bottom and instrument cassette carrier is critical for a proper instrument run. Any debris or lint can interfere with image acquisition.

10x GENOMICS

Getting Started

Instrument Setup

Prior to starting an experiment on the Xenium Analyzer, a series of steps must be performed to ensure proper function. The following section describes the process required to get started on the instrument.

Warning: Avoid using the Xenium Analyzer in a manner not specified by 10x Genomics. *The Xenium Analyzer 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.

Required for First-time Use Only

- Register the instrument to 10x Genomics Cloud.

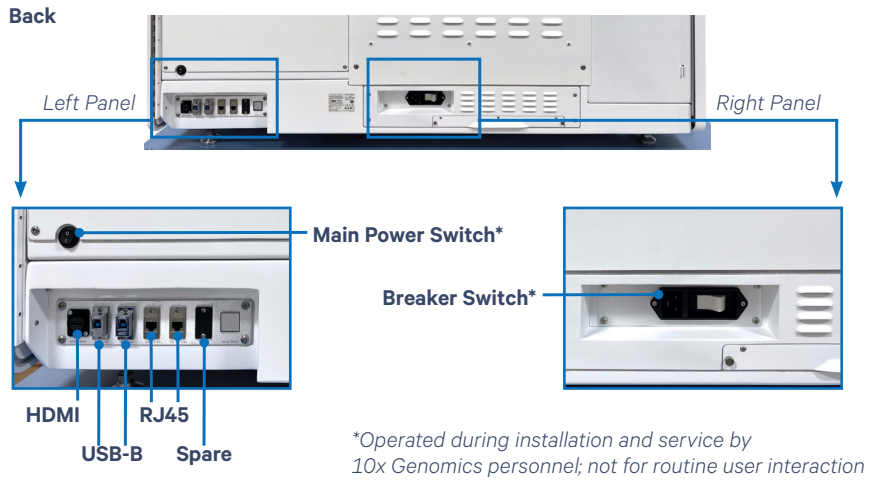
Instrument Setup
contd.

The user should not operate the switches at the back panel. The breaker switch and the main power switch on the back panel will be activated/used only during instrument installation and service.

Turn on the System

a. Power on the instrument using the power button (press >3 sec) on the side panel (right). See detailed information below.

- **Breaker Switch** (back right panel): Activated during installation. Not for routine user interaction. Should be kept in ON position ("I" pushed in) for normal operation.
- **Main Power Switch** (back left panel): Activated during installation. Not for routine user interaction. Should be kept in ON position ("I" pushed in) for normal operation.
- **Power Button** (side right panel): Only active when Breaker and Main Power Switch are ON (Blue LED light will be illuminated). Press the power button for **>3 sec** to initiate Xenium Analyzer and Xenium Analysis Computer power ON mode. Wait 3 minutes after powering ON before proceeding.

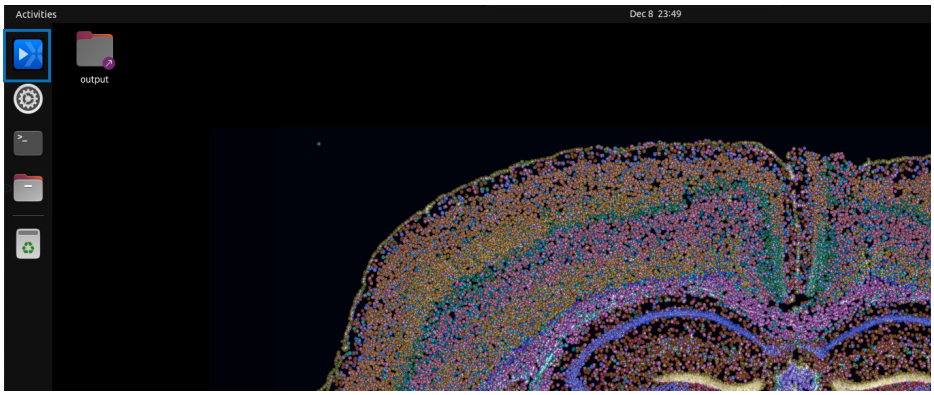


b. After the instrument powers on (~few minutes), login by selecting "Xenium User" on the touchscreen and enter password.

For first time users, a password for the user account on each instrument will be provided by 10x Genomics when the instrument is shipped. Contact support@10xgenomics.com for guidance regarding changing the password.

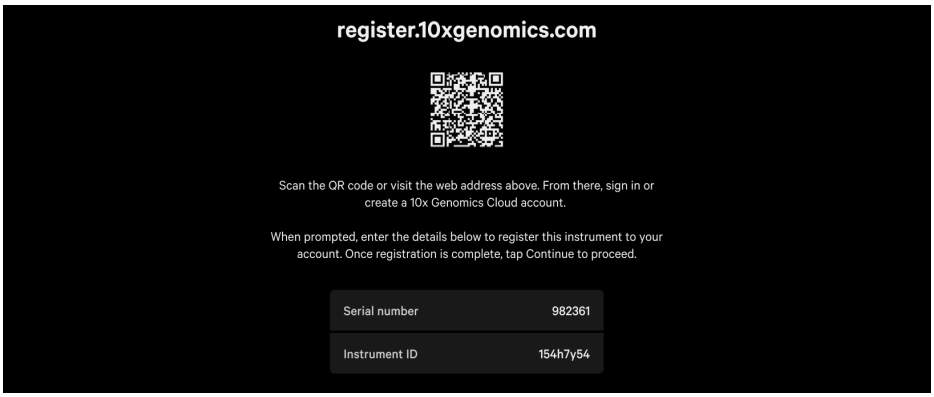
Instrument Setup
contd.

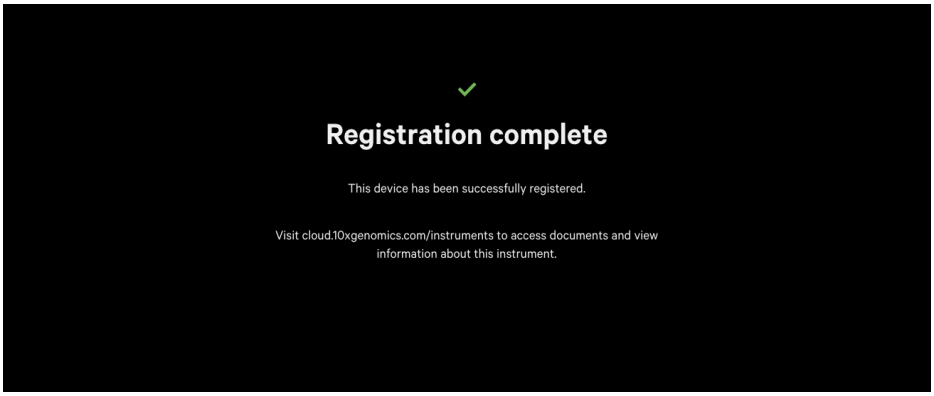
c. Start the Xenium Analyzer Application by clicking the blue icon on the touchscreen.



Registering the Instrument (First-time Use Only)

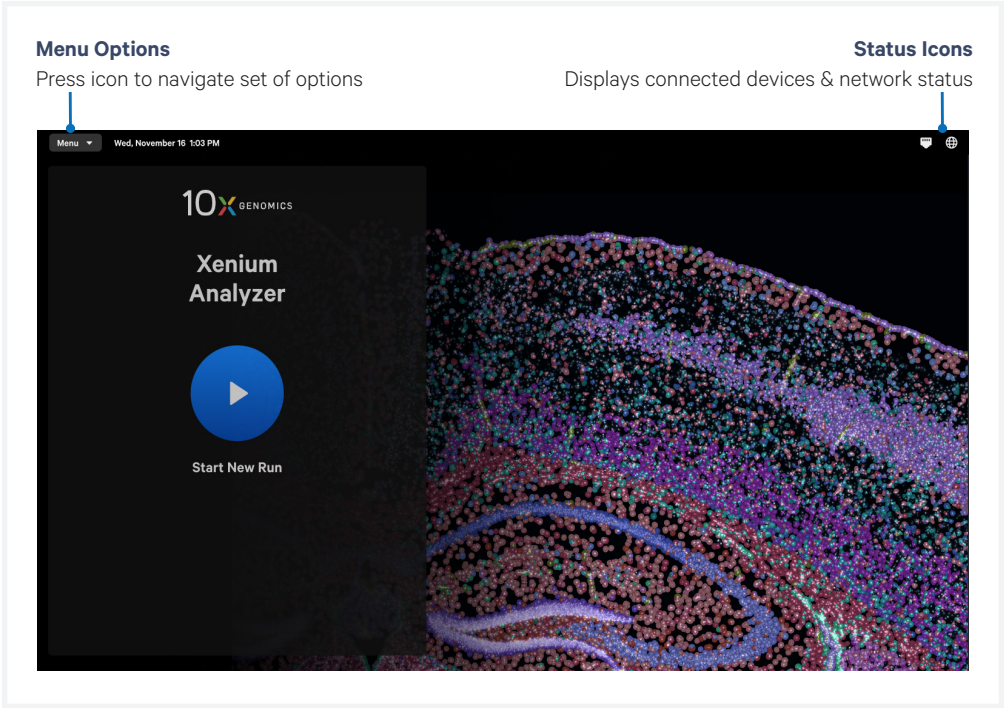
a. Upon initial opening of application, a registration screen will appear. Follow the onscreen instructions to register the instrument to the 10x Genomics Cloud. When the instrument is successfully registered, the instrument home screen will appear.





Touchscreen Menu Options

The instrument home screen enables starting a run and accessing the Menu Options (top left corner of the screen) for Settings and Contact Support information.



Instrument Data and Settings

Run data, readiness testing, and system information can be found under the Settings menu.

Touchscreen Menu Options *contd.*

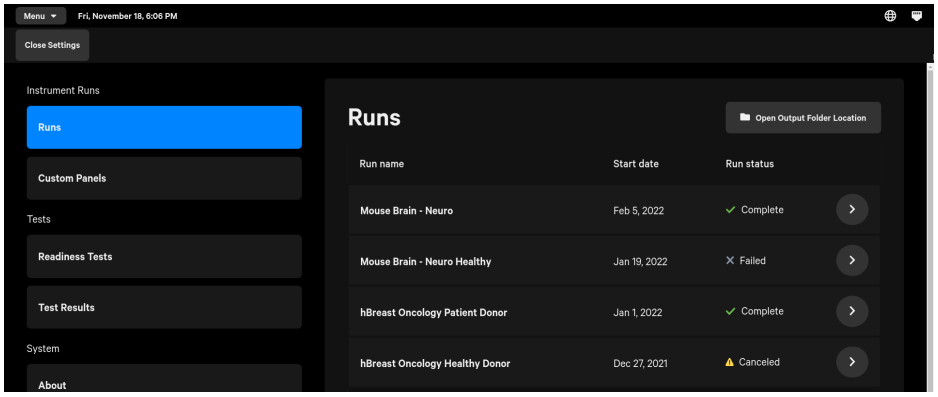
Run Data

- Runs: A list of runs performed on the instrument will appear here, including start date and run status. Completed runs with analysis outputs are marked as "Complete". Output data from each run can be accessed here. For more detailed information on how to handle data post-run, refer to the Data Output chapter.
- Custom Panels: View and manage custom or add-on panels that have been uploaded to the instrument here. Custom panels cannot be added on this screen and must be uploaded during the run setup.

Instrument Runs

Runs stored on the instrument appear here.

Any deleted data will not be visible.



Tests

- Initiate instrument tests and view test results here. The only user run test is the Readiness Test. For detailed information on when and how to run a readiness test, refer to the Readiness Test section.

Additional types of readiness tests available on the instrument are only to be launched by 10x Field Engineers.

System

- Information about the system (including instrument serial number), analytics and privacy, and software versions are found here.

To exit settings, click the "Close" button on the top left corner of screen, or select Menu drop down at the top left and select Close settings.

Network Connectivity

Xenium Analyzer has a highly interactive user interface paired with network connectivity, intended to provide a seamless user experience along with efficient remote monitoring to optimize instrument performance. This also gives 10x Genomics the ability to respond quickly and troubleshoot any issues that may occur.

Refer to the Xenium Analyzer Network Connectivity Guidelines Techinical Note (CG000645) for comprehensive information regarding remote performance monitoring and remote support along with additional technical details.

Software Updates
for Versions 1.6 +

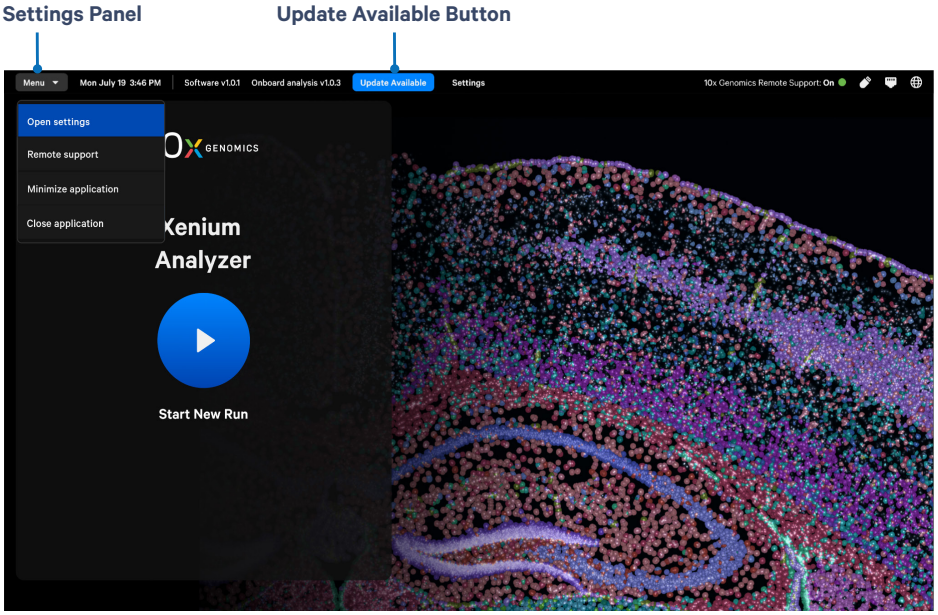
Xenium Analyzer Application should be kept up to date to ensure instrument can utilize the latest assay products, features, and bug fixes. Software updates may improve or alter the on-instrument analysis pipeline. Changes to the pipeline may introduce batch effects if upgrades are completed between runs involved in a multi-run experiment.

Refer to the Xenium Software Release Notes for details before completing Software Updates.

Instrument must be connected to the Internet to receive update notifications and perform installation.

Initiate Software Update

There are two ways to initiate a software update: from the home screen, or from the Software section of the Settings panel.



From Home Screen

a. When software update is available, a blue “Update Available” button is visible on the top bar. Click Update Available button. A popup window will appear indicating the version of available upgrade and link to software release notes.

b. Click download and install to continue with software update.

Software Updates
contd.

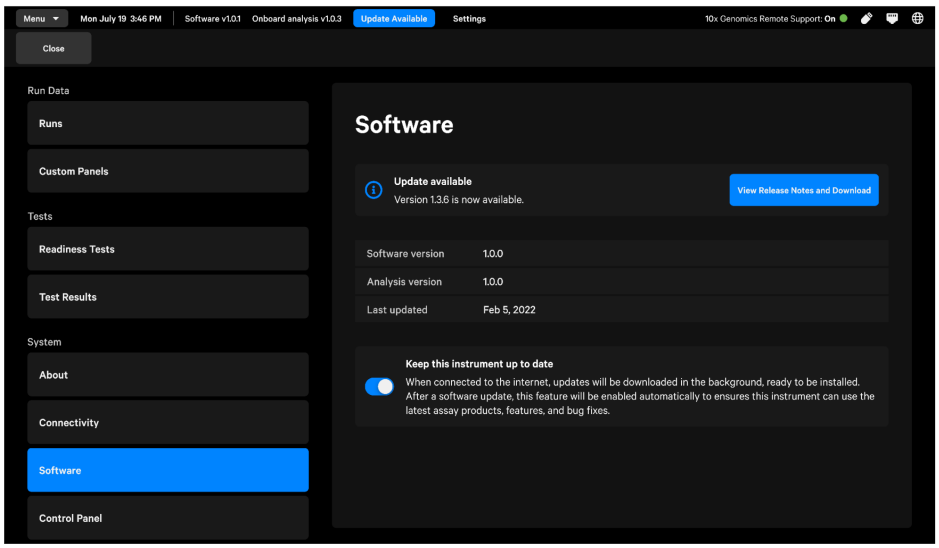
From Settings Panel

a. On the top left corner of the screen, click Menu -> Settings -> Software

b. When software update is available, screen will say "Update available". Click View Release Notes and Download to download update. A popup window will appear indicating the version of available upgrade and link to software release notes.

c. Ensure "Keep this instrument up to date" is toggled ON to have updates downloaded in the background. Using this feature will not impact instrument runs.

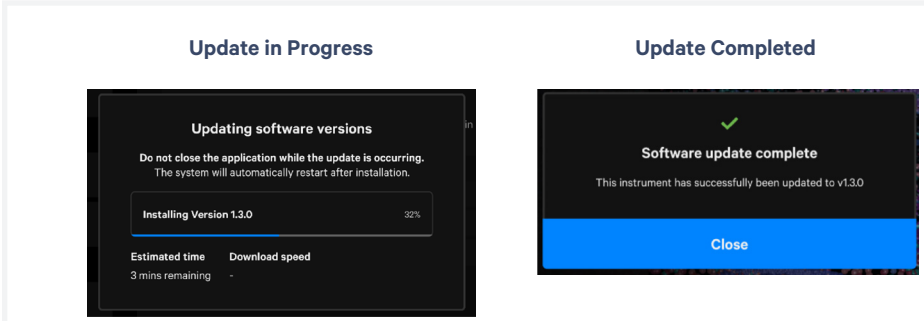
TIPS *When turned ON, updates will be downloaded in the background when available to ensure instrument remains up to date. User must still install new version.*



d. Click download and install to continue with software update.

Software Updates
contd.

Installing Software Update



e. During installation, a popup window will appear showing progress bar with completion percentage, estimated time remaining, and current download speed.

f. Following installation, the instrument will be automatically rebooted and login is required.

g. Upon opening Xenium Analyzer software, a popup window will display Software update complete, indicating successful update.

Readiness Test

The Readiness Test verifies that all systems are working optimally and the instrument is ready for use. No reagents are used (the Readiness Test is the only dry run the instrument utilizes). The Readiness Test is included as a pre-run verification for all instrument runs, but can be initiated as a standalone operation at the discretion of the end user from the Tests Menu option.

a. To initiate the Readiness Test, select Menu (top left corner of screen) and choose Open Settings on the touchscreen.

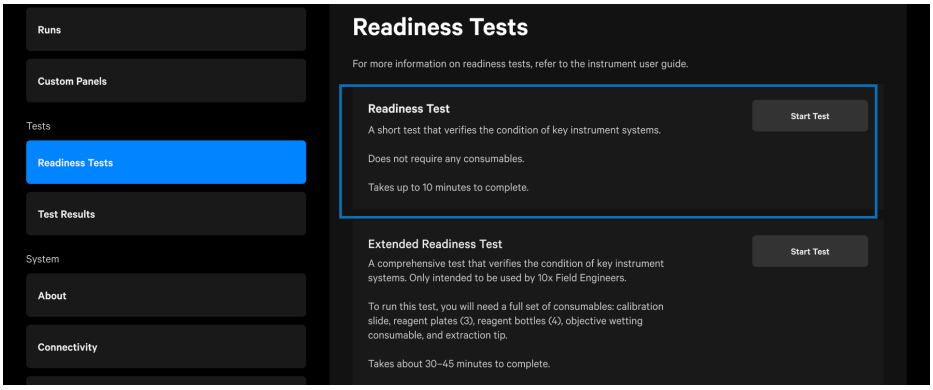
b. Under the Tests category, select Readiness Tests.

c. Three types of Readiness Tests will appear. The first option, Readiness Test, is the option for end users to run. To start the test, click "Start Test" under Readiness Test.

Additional types of readiness tests available on the instrument are only to be launched by 10x Field Engineers.

Readiness Test

Select "Readiness test" to check instrument parameters



d. A successful Readiness Test verifies the instrument is ready for use. Follow onscreen instruction in the event of a failed or incomplete test.

e. Exit Readiness Tests screen by selecting the Close settings button at the top left corner of the screen, or select the Menu drop down and click Close settings. The home screen will appear following exiting.

Protocol Steps & Timing

(on-instrument; for both FFPE & FF samples)

Steps	Timing	
	Hands-on Time	Total Time
Day 1		
Thaw Decoding Reagent Module B	5 min	16-72 h at 4°C*
Day 2		
Prepare Buffers	1 h	1 h
Initialize Instrument	-	5-10 min
Input Experimental Details	5-10 min	5-10 min
Load Instrument	~5 min	~5 min
Sample Scan	-	1 h
Select Region & Initiate Run	~10 min	~10 min
Day 4-6		
Run Time	-	2-4 days
Post-Run Cleanup	5 min	10 min

**2.5 h at 37°C water bath for same day use*

Reagent Preparation

Step 1: Reagent Plate Preparation

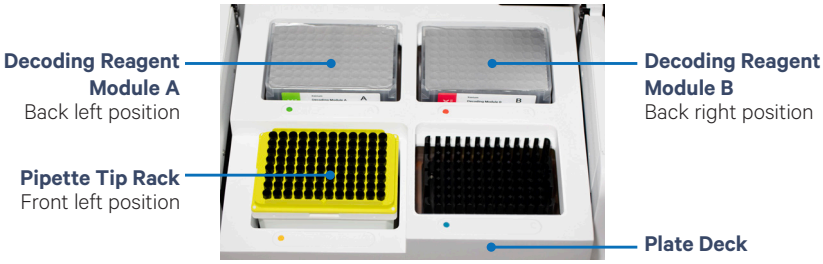
Overview

The following section describes how to prepare reagent plates (includes two modules: Decoding Reagent Module A and Decoding Reagent Module B), prior to an experiment run. Follow the instructions outlined below for proper handling.

Placement of Reagent Plates in Xenium Instrument

(load only when prompted on the instrument touchscreen)

Reagent Plate Positions on the Deck



Gather Items

Gather the items listed in the table below. For items with multiple options listed, choose an option based on availability and preference.

TIPS **Decoding Reagent Module B requires overnight thawing at 4°C. Ensure plate is removed from -20°C and placed at 4°C the night prior to the instrument run.**

Refer to SDS for instructions on proper handling and disposal of volatile and hazardous chemicals.

GET STARTED! Item		10x PN	Preparation & Handling	Storage
☐	Xenium Decoding Reagent Module A	1000624	-	4°C
☐	Xenium Decoding Reagent Module B	1000625	Thaw in sealed mylar bag at 4°C for 16-72 h or at 37°C for 2.5 h	-20°C
	Additional Materials			
☐	Empty plate (deep-well, 96 well) for counterbalancing and centrifuge that fits deep-well 96 well plates (~2 ml vol.) *(Allegra® X-14 Series Benchtop Centrifuge 120 V or equivalent)*			
☐	Serological Pipettes			
☐	Plate seal			
☐	Laboratory Balance			

This list may not include some standard laboratory equipment.

Step 1: Reagent Plate Preparation *contd.*

A Xenium Decoding Reagent Module A

Decoding Reagent Module A is oxygen sensitive! Keep plate in its original vacuum sealed mylar packaging during storage at 4ºC.

a. On the day of the instrument run, open the mylar packaging and remove plate with the intact foil seal. The foil seal on the plate should not be removed at any time. Mix by gently inverting the plate 20x without introducing bubbles. DO NOT vortex. Maintain on ice.

Plate must be used within 5 days (includes run time) after opening and removal from mylar packaging

b. Prepare a plate for counterbalancing as described in the Plate Counterbalancing Instructions on the next page.

c. Place the reagent plate and the plate for counterbalancing in a swinging bucket centrifuge. Once balanced, centrifuge at **300 rcf** for **1 min** at **room temperature**.

d. Remove from centrifuge and place plate at **4ºC** until loading. DO NOT invert the plate after centrifugation.

B Xenium Decoding Reagent Module B

Keep Decoding Reagent Module B in its original vacuum sealed mylar packaging during storage at -20ºC and during thaw at 4ºC.

a. Thaw Decoding Reagent Module B in its original packaging at **4ºC** for **16-72 h** or at **37ºC** for **2.5 h**. Unopened plate in its original mylar packaging may be kept at **4ºC** for up to **3 days**.

b. Remove thawed plate and equilibrate at room temperature for **30 min**.

c. Open the mylar packaging to remove plate with the intact foil seal. The foil seal on the plate should not be removed at any time. Mix by gently inverting the plate 20X without introducing bubbles. DO NOT vortex. Maintain at **room temperature**.

d. Prepare a plate for counterbalancing as described in the Plate Counterbalancing Instructions on the next page.

e. Place the reagent plate and the plate for counterbalancing in a swinging bucket centrifuge. Once balanced, centrifuge at **300 rcf** for **1 min** at **room temperature**.

f. Remove from centrifuge and leave plate at **room temperature** until ready to load. DO NOT invert the plate after centrifugation.

Step 1: Reagent Plate Preparation *contd.*

Reagent Plate Preparation Summary		
Step	**A Xenium Decoding Reagent Module A**	**B Xenium Decoding Reagent Module B**
Thaw	-	Store in the sealed mylar bag at: **4°C** for **16-72 h** OR **37°C** water bath for **2.5 h**
Day of instrument run	*(Oxygen sensitive)* Remove plate from **4°C** Open the mylar packaging to remove plate *(foil seal stays intact)* Mix by gently inverting the plate 20x without introducing bubbles. DO NOT vortex Maintain on **ice**	 Remove plate from **4°C**. Equilibrate at **room temperature** for **30 min** Open the mylar packaging to remove plate *(foil seal stays intact)* Mix by gently inverting the plate 20x without introducing bubbles. DO NOT vortex Maintain at **room temperature**
Counterbalance	Prepare counterbalancing plate	Prepare counterbalancing plate
Centrifuge	**300 rcf** for **1 min** at **room temp.**	**300 rcf** for **1 min** at **room temp.**
Before loading	Maintain at **4°C**	Maintain at **room temperature**

Plate Counterbalancing Instructions

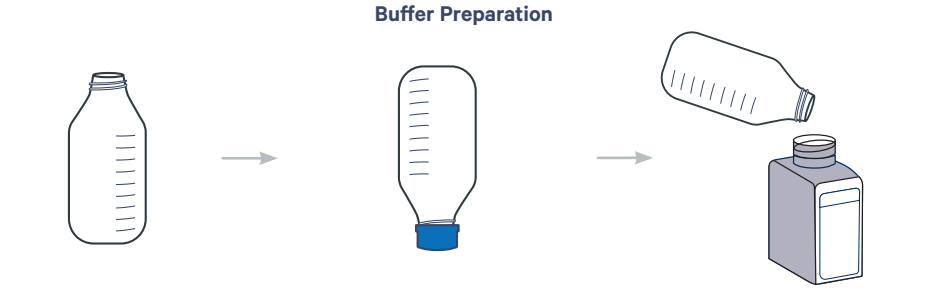
Xenium Decoding Reagent Module A and Decoding Reagent Module B do not weigh the same and should be counterbalanced separately

- Weigh the Xenium module plate with elastic and lid on.
  (example: 190 g)
- Place the empty counterbalancing deep-well 96 well plate on the weighing balance and using a pipette (multichannel/serological) add water to the plate wells until the total weight is equal to the Xenium module plate ± 1 g.
  (example: counterbalancing plate with water=189.6 g)
- Remove from the counterbalancing plate from the weighing balance, add a seal to it, and use for counterbalancing the Xenium module plate.

Step 2: Buffer Preparation

Overview

This section provides guidance on the preparation of Xenium Reagent Bottle buffers for use in the Xenium Analyzer. Reagent Bottle buffers should be prepared fresh before use. Ensure buffers are transferred to the correct Reagent Bottle as indicated in the instructions on the following pages. An overview of the buffer preparation process is outlined below.



Placement of Reagent Bottles in Xenium Instrument

(load only when prompted on the instrument touchscreen)

Reagent Buffers Bottles

Label Color	Bottle Position	Reagent Buffer Bottle Name	Composition
	1	Deionized Water/Xenium Instrument Wash Buffer	Milli-Q Water
	2	Xenium Sample Wash Buffer A	PBS + Tween
	3	Xenium Sample Wash Buffer B	Milli-Q Water
	4	Xenium Probe Removal Buffer	DMSO + Tween + KCl

Step 2: Buffer Preparation *contd.*

Gather the items listed in the table below. For items with multiple options listed, choose an option based on availability and preference.

GET STARTED! Item		10x PN	Preparation & Handling	Storage
☐	Deionized Water/ Xenium Instrument Wash Buffer*	3001198	-	Ambient
☐	Xenium Sample Wash Buffer A*	3001199	-	Ambient
☐	Xenium Sample Wash Buffer B*	3001200	-	Ambient
☐	Xenium Probe Removal Buffer*	3001201	-	Ambient

**Empty bottles*

Refer to SDS for instructions on proper handling and disposal of volatile and hazardous chemicals.

	Additional Materials
☐	Nuclease-free Water (not DEPC-treated)
☐	PBS-Tween
☐	PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free and Tween 20 Detergent Solution (10% solution)
☐	100% DMSO
☐	Potassium Chloride (KCl)
☐	Glass Bottles with Cap (500 ml, 1 L)
☐	Serological Pipettes (10 ml, 25 ml, 50 ml) & Serological Pipette Controller
☐	Pipette Tips (1,000 µl) & Pipette
☐	Ultrapure/Milli-Q water

Choose only one for Xenium Sample Wash Buffer A

This list may not include some standard laboratory equipment.

Step 2: Buffer Preparation *contd.*

Prepare Reagent Bottle Buffers

Prepare the following buffers fresh prior to setup of the Xenium Analyzer. Read all the preparation instructions for various options before proceeding with buffer preparation.

Prior to preparation, sterilize glass bottles by autoclaving. Ensure bottles and caps are free of residual detergents, debris, and nuclease activity is minimized. Measure liquids using a graduated cycler for accuracy. A funnel may be used when pouring buffers. Ensure buffers are free of particulate material as that can clog the instrument lines.

1 Deionized Water/Xenium Instrument Wash Buffer

Fill Reagent Bottle #1 with **500 ml** of Milli-Q Water using a graduated cylinder to make Xenium Instrument Wash Buffer and cap bottle to close (use the standard cap and not the Xenium Buffer Cap).

2 Xenium Sample Wash Buffer A

Prepare Xenium Sample Wash Buffer A in a 1 L glass bottle based on either option A, B, or C, followed by the steps listed below.

Pick only one of the three options to prepare the PBS-T.

Option A: Use PBS-Tween (PN P3563-10PAK):

Xenium Sample Wash Buffer A *Add reagents in the order listed*	**PN**	**1X (amount)**
Nuclease-free Water	AM9932 or CDUFBI0A1	1 L
PBS-Tween	P3563-10PAK	1 Pack
Total	—	**1 L**

Option B: Use PBS-Tween (PN PPB005-20PAK):

Xenium Sample Wash Buffer A *Add reagents in the order listed*	**PN**	**1X (amount)**
Nuclease-free Water	AM9932 or CDUFBI0A1	1 L
PBS-Tween	PPB005-20PAK	2 Packs
Total	—	**1 L**

Option C: Use 10X PBS and 10% Tween:

Xenium Sample Wash Buffer A *Add reagents in the order listed*	**PN**	**1X (amount)**
Nuclease-free Water	AM9932 or CDUFBI0A1	895 ml
10X PBS	AM9624	100 ml
10% Tween 20	28320	5 ml
Total	—	**1 L**

Step 2: Buffer Preparation *contd.*

If using Option A or B (PBS-T powder bags):

a. Using a graduated cylinder, add **1 L** nuclease-free water to a 1 L glass bottle.

b. Add appropriate number of PBS-Tween powder bags to the **1 L** nuclease-free water.

TIPS *Before opening the bag with the powder, ensure that the powder is collected at the bottom of the bag.*

c. Proceed to step d.

If using Option C (preparing from 10X PBS and 10% Tween):

a. Using a graduated cylinder, add **895 ml** nuclease-free water to a 1 L glass bottle.

b. Using a graduated cylinder, add **100 ml** 10X PBS to the glass bottle.

c. Using a serological pipette, add **5 ml** Tween-20 to the glass bottle. Ensure that Tween-20 is completely dispensed. Rinse the pipette by pulling in **~5 ml** PBS from the bottle and dispensing the entire volume back into the bottle.

d. Cap bottle and slowly invert approximately 10x. Ensure that minimal bubbles are introduced during inversion process.

DO NOT use magnetic stir bars or vigorous shaking to dissolve the PBS-T

e. Pour prepared buffer into Reagent Bottle #2 and cap the bottle (use the standard cap and not the Xenium Buffer Cap). If bubbles are created during mixing, wait **~30 min** or until the bubbles dissipate. Minor amount of bubbles are acceptable.

Step 2: Buffer Preparation *contd.*

3 Xenium Sample Wash Buffer B

a. Fill Reagent Bottle #3 with **500 ml** of Milli-Q Water using a graduated cylinder to make Xenium Sample Wash Buffer B and cap the bottle (use the standard cap and not the Xenium Buffer Cap).

4 Xenium Probe Removal Buffer

Prepare **300 ml** Xenium Probe Removal Buffer (1X) in a 500 ml glass bottle according to the table and steps listed below.

Xenium Probe Removal Buffer *Add reagents in the order listed*	PN	Stock	Final	1X (ml)
Nuclease-free Water		—	—	139.5
DMSO *DMSO is hazardous and should be added to nuclease-free water inside a fume hood. Refer to SDS for instructions on proper handling and disposal. Mix after adding DMSO.*		100%	50%	150
KCl		2,000 mM	50 mM	7.5
Tween 20		10%	0.1%	3
Total	—	—	—	**300**

a. Add nuclease-free water to glass bottle using a 50 ml serological pipette.

b. Add DMSO to the nuclease-free water using a 50 ml serological pipette.

DMSO is hazardous and should be added to nuclease-free water inside a fume hood. Refer to SDS for instructions on proper handling and disposal.

c. Cap bottle and invert 10x to mix. Note that the buffer may become warm during preparation.

d. Pipette KCl into glass bottle using a 10 ml serological pipette.

e. Slowly pipette Tween 20 into glass bottle using a p1000 pipette. Ensure all the Tween 20 is dispensed from the pipette tip.

f. Cap bottle and slowly invert approximately 10x. Ensure that no bubbles are introduced during the inversion process.

TIPS *Tween 20 may cause the Xenium Probe Removal Buffer to appear cloudy.*

g. Pour prepared buffer into Reagent Bottle #4 and cap the bottle (use the standard cap and not the Xenium Buffer Cap). Maintain the buffer at room temperature for **30 min** to cool it down and to clear bubbles created during mixing. Minor amount of bubbles are acceptable.

10x GENOMICS

System Operation

Initialize Instrument

Initialize ~10-20 min › Load ~5 min › Sample Scan 1 h › Region Sel. ~10 min › Run 2-4 days › Cleanup ~5 min › Unload ~10 min

a. Turn on the instrument using the power switch at the side panel (right) of the instrument.

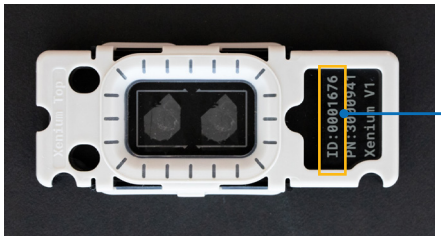
b. Click "Start New Run" button on the instrument home screen.

- A series of automated system checks will be performed by the instrument to verify that all systems are working correctly (~3 min).

c. Input Run Name. The Run Name will be used in the Analysis Summary to refer to this particular run. Run name can contain maximum 33 characters and cannot contain !@#$%&*)+=

d. Enter the following details on the instrument screen. If using only one slide, either of the two cassette carriers may be used.

- **Cassette Name** (used to reference data from this cassette) Cannot contain !@#$%&*)+=
- **Xenium Slide ID** (7-character ID found on the bottom short edge of the slide)

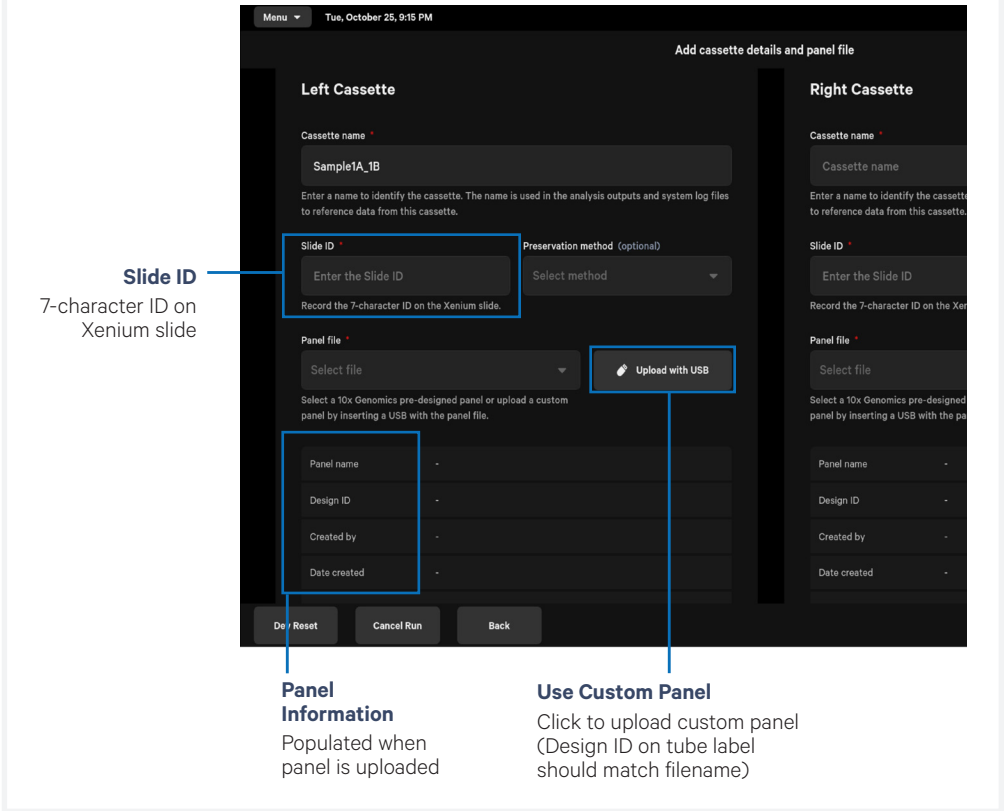


- **Panel File** (select a 10x Genomics pre-designed panel)
  If using a compatible custom gene panel, upload the custom panel file by inserting a USB (containing the panel file) in the Xenium Analysis Computer USB port.

If using a custom panel, the Design ID on the label of the tube containing the custom panel should match with the first portion of the custom gene panel electronic file name.

TIPS *The Xenium Analyzer is compatible with exFAT file systems. See Data Output chapter for additional details.*

Initialize Instrument *contd.*



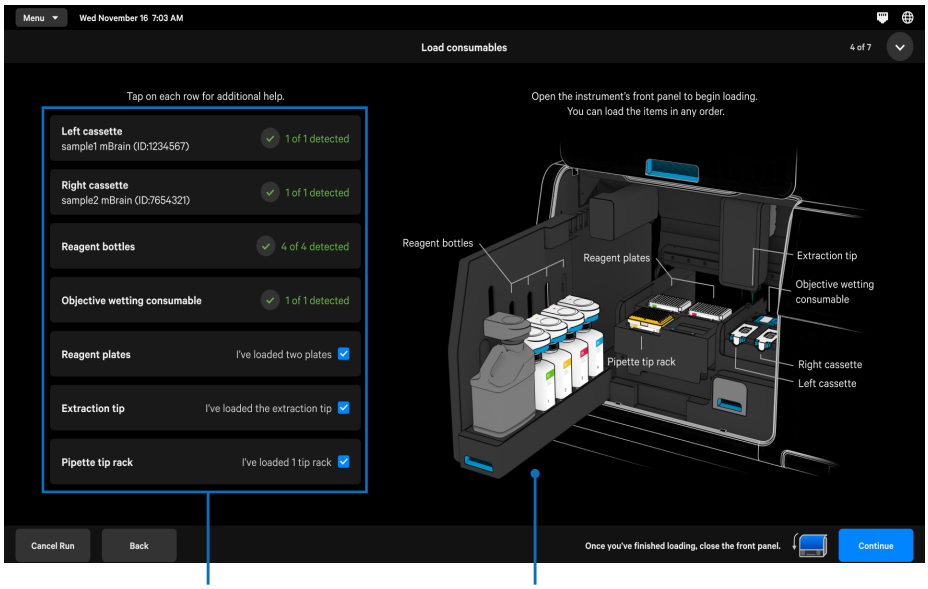
e. When all the information is populated, click “Continue”.

Load Consumables

Initialize ~10-20 min › **Load ~5 min** › Sample Scan 1 h › Region Sel. ~10 min › Run 2-4 days › Cleanup ~5 min › Unload ~10 min

See Troubleshooting section for guidance if any errors occur during loading consumables.

Open the front panel. Follow touchscreen instructions to properly load consumables. Load in the order specified on the touchscreen.



Consumables
Loading status will appear here

Deck Directory
Displays location of consumables to aid in loading

To aid in efficient and complete loading, the status of each item is shown on the left side of the screen and location of consumables within the instrument deck is depicted on the right.

Gather all the items listed below and load them as per instructions provided. All the items must be loaded correctly to begin the instrument run.

TIPS *Click each row on the touchscreen to view detailed help and instructional animations.*

- Objective Wetting Consumable*
- Reagent bottles with buffer (Reagent Preparation section) + Xenium Buffer Caps*
- Reagent Plates (Reagent Preparation section)
- Pipette Tip Rack*
- Extraction Tip*
- Cassette/s (with tissue sections on the Xenium Slide ready for the instrument run)

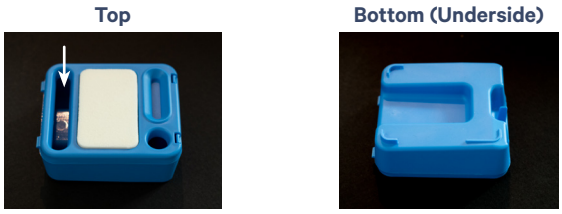
**In Xenium Decoding Consumables Kit, PN-1000487*

Load Consumables *contd.*

Objective Wetting Consumable

a. Place a new Objective Wetting Consumable behind the cassette carrier with the reagent priming reservoir on the left.

Loading the Objective Wetting Consumable



The bottom of the Objective Wetting Consumable is molded to the instrument, therefore Objective Wetting Consumable can only be placed in one orientation. Place Objective Wetting Consumable in instrument with the reagent priming reservoir on the left (white arrow).

Reagent Bottles

a. Uncap the reagent bottles that were prepared earlier in the Buffer Preparation section. Recap each bottle with a Xenium Buffer Cap (included in the Xenium Decoding Consumables kit). This cap is required for loading onto the instrument.

Xenium Buffer Cap

(included in the Xenium Decoding Consumables kit)

For each reagent bottle, replace the standard reagent bottle cap with a Xenium Buffer Cap prior to loading onto the instrument

Load Consumables *contd.*

b. Place each bottle within the bottle carrier in the designated order.

Bottle position is color and number coded. Color is indicated on top of bottle carrier cap and number is labeled on the bottle carrier itself. Match bottle position color and number with label on reagent bottle for accurate placement. Incorrect placement will result in a failed instrument run.

Color *(on Bottle and Instrument)*	Position in Bottle Carrier	Reagent Bottle Buffer
	1	Deionized Water/Xenium Instrument Wash Buffer
	2	Xenium Sample Wash Buffer A
	3	Xenium Sample Wash Buffer B
	4	Xenium Probe Removal Buffer

c. Push the bottle carrier caps down to the top of the bottles to seal.

TIPS *If the instrument screen does not show the presence of the loaded bottles, use a firm downward pressure on the bottle carrier caps to enable detection.*

d. Ensure that the Waste Bottle on the left side of the instrument has been emptied and the empty uncapped Waste Bottle has been placed in the first position (closest to user). Push the bottle carrier cap down to the top of the Waste Bottle to seal.

e. Push the bottle carrier back into place.

Load Consumables *contd.*

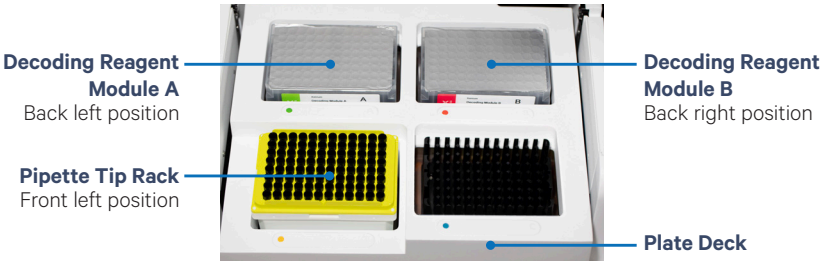
Reagent Plates

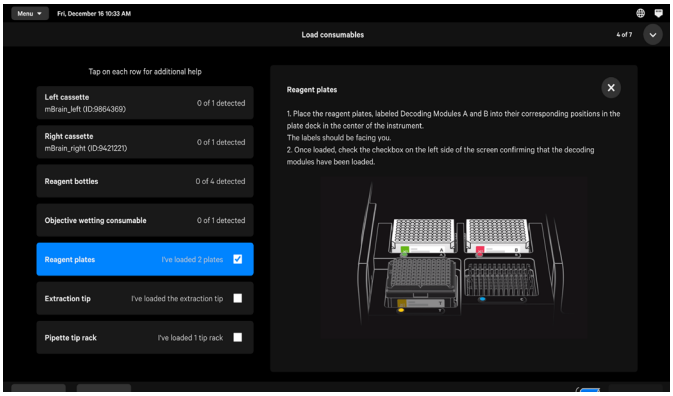
a. Remove elastic and lid from reagent plates.

b. Place the reagent plates (prepared earlier in the Reagent Plate Preparation section) labeled Decoding Reagent Module A and B into the plate deck in the center of the instrument with the plate label and notches facing the user. Firmly press down to make sure module A and B are properly positioned on the deck

- Decoding Reagent Module A position = back left. Plate is labeled A.
- Decoding Reagent Module B position = back right Plate is labeled B.

Reagent Plate Positions on the Deck





Confirm that the plate labels face the user when placed into deck and the correct plate is placed in the correct location. Improper placement will result in a failed expt. run.

c. Once loaded, check the box on the touchscreen confirming that the decoding reagent modules (reagent plates) have been loaded.

Load Consumables *contd.*

Pipette Tip Rack

a. Place a new pipette tip rack (after removing the lid) directly into the lower left position on the plate deck (directly in front of Decoding Reagent Module A plate) with the label facing the user.

b. Push down into place to secure. Pipette tip rack should sit flat in plate deck. If it is slanted or not secure, remove and place again. Remove the pipette tip rack lid.

TIPS *The bottom of the pipette tip rack position is lined with a black mold. This aligns with the bottom of the pipette tip rack. Tip rack should fit flat and snug in place when proper alignment is achieved.*

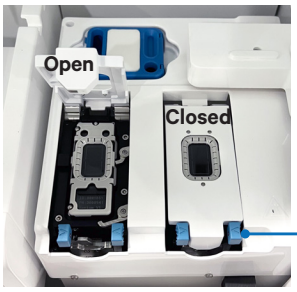
Extraction Tip

a. Align Extraction Tip into extract axis head and push tip up firmly. The tip should fit securely on and not feel loose or fall out.

Cassette

a. Squeeze the release buttons to unlatch the cassette carrier. The right button will move while the left button is static.

Open the Xenium Analyzer Cassette Carrier



Press Buttons
Open cassette carrier by squeezing the two blue release buttons; (right button moves, left button is static)

b. Clean the cassette carrier. Spray 70% isopropanol onto a lint-free laboratory wipe and clean the surface of the carrier, paying attention to the raised areas that come into contact with the slide. Let evaporate.

- Optional: Spray 70% isopropanol onto a cotton swab and use to clean off crevices if necessary.

c. Use compressed air to remove any remaining lint paying close attention to raised areas. Confirm surface is dry and free of lint.

d. Retrieve the assembled Xenium Slide Cassette(s) (processed as per Xenium In Situ Gene Expression User Guide CG000582).

e. Check assembled cassette to ensure the seals are not leaking liquid.

See the Troubleshooting section to fix leaking cassette assembly.

Load Consumables *contd.*

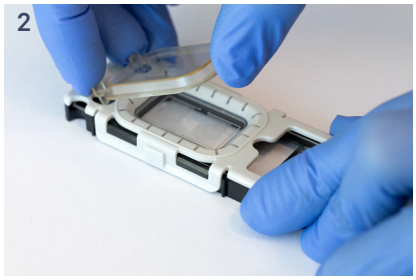
f. Clean the bottom of the slide surface with 70% isopropanol using a lint-free laboratory wipe without spilling the storage buffer. Confirm the bottom of the slide bottom is clean and dry.

g. Confirm that the slide ID on the slide matches the ID number shown on the touchscreen.

The lid may be saved for using later if storing the cassette assembly post-run.

Xenium Cassette Lid Removal

Push the upper two tabs with index and middle fingers and lower tab with thumb.



While maintaining inward pressure, pull upward with thumb until lower clip disengages.

A dry, clean, lint-free surface on both the slide bottom and instrument cassette carrier is critical for a proper instrument run. Any debris or lint can interfere with image acquisition.

h. Prior to loading, remove the lid from the cassette. DO NOT spill or remove PBS-T covering the slide to ensure that the sections do not dry up.

i. Open the cassette carrier lid fully. Place the cassette into the carrier. The top left notch on the assembled cassette will align with the instrument. Be sure the assembled cassette is lying completely flat in the carrier.

Failure to open the cassette carrier lid fully and placing assembled cassette into carrier improperly will result in breaking the slide when the lid is closed. Ensure proper loading before closing cassette carrier lid to avoid damage.

Proper Loading of Assembled Cassette in Instrument

Notch Alignment
Align notches on slide cassette with raised areas on the instrument (3 areas denoted by blue circles)

Carrier Lid Open
Open carrier lid fully before loading slide cassette

Slide Cassette Completely Flat
Ensure slide cassette is sitting flat before closing

Load Consumables *contd.*

j. Close the cassette carrier lid until it clicks into place.

k. Repeat for the Right Cassette.

Also Check:

Waste Tip Tray: Ensure that the Waste Tip Tray has been emptied. To empty the tray, slide the waste tip drawer open, empty the tray, place it back in the tray and push the waste tip drawer to close.

Once all consumables are loaded, close the front panel of the instrument.

- Confirm that all consumables are loaded correctly on the touchscreen. Green check marks next to consumable indicates successful loading.
- User must manually select the check box on the touchscreen for Pipette tip rack, Reagent Bottles, and Extraction tip to confirm before proceeding to the next step.
- Click "Continue" when complete.

The instrument will verify that all consumables are loaded properly before proceeding to the next step.

If any consumable is not detected, an error message will appear. To address, open the front panel, reload necessary consumable(s), close the front panel and click "Continue".

Only the presence or absence of consumable is detected. Correct placement for reagent bottles and reagent plates in the right locations is not detected. Double check the correct placement of these consumables before continuing. Improper placement will result in a failed instrument run.

See the Troubleshooting section for guidance on resolving errors during loading consumables; includes guidance on how to open the front panel to access the instrument deck prior to starting a run.

Sample Scan

Initialize ~10-20 min › Load ~5 min › **Sample Scan 1 h** › Region Sel. ~10 min › Run 2-4 days › Cleanup ~5 min › Unload ~10 min

a. Following successful loading, the instrument will begin Sample Scan during which an initial image of each slide is taken by the instrument. The image will be used for region selection.

Xenium Analyzer is sensitive to vibration. Ensure sources of vibration are kept away from the instrument during the scan.

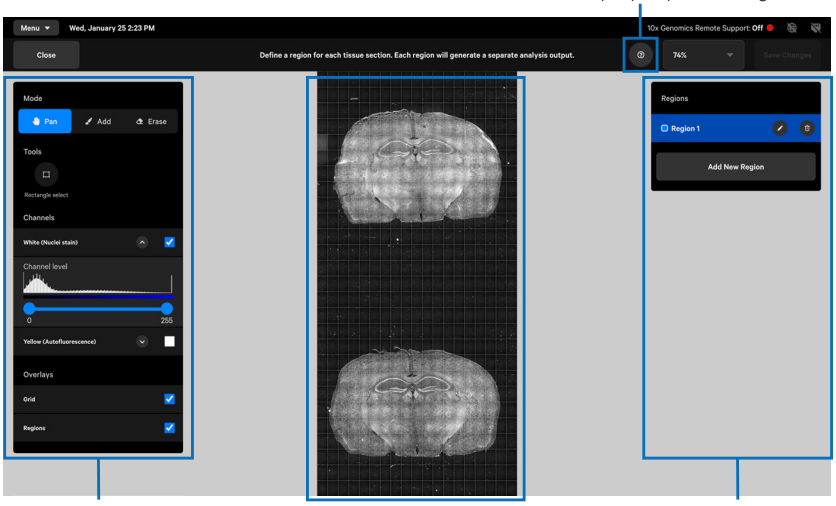
DO NOT interact with the instrument during the scan; avoid touching the keyboard and trackpad. The front panel of the instrument remains locked during and after Sample Scan.

b. Once Sample Scan (**~1 h**) is complete, click "Continue". The sample area and related options are shown after overview scan is complete.

TIPS *Fine-tuning and reviewing sample scan image at this step will help with region selection.*

Overview Scan
Layout and features seen following completion

Region Selection Guidance
Instructions to properly select regions



Options Panel
Toggle views on/off and adjust channels

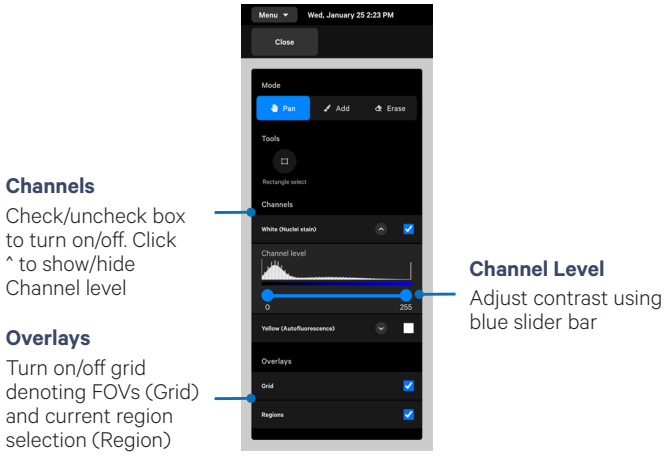
Sample Area
Shows image of scanned sample

Region Information
View, add, edit or delete regions

c. Review sample scan image. Use the panel on the left hand side of the screen to turn on/off channels, adjust channel levels, and view overlays.

Overview Scan Image Options

Left panel on screen after overview scan is completed

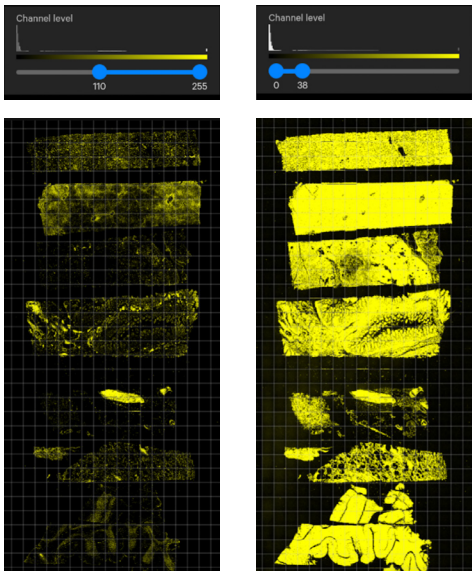


TIPS

To better define tissue morphology prior to region selection, fine-tune intensity and toggle channels on/off as needed. Histogram shows number of pixels at different intensity values. Adjust slider to optimally gate a suitable threshold for pixel intensity. Tissue can appear different depending on the threshold.

Channel Levels

Adjusting channel levels can alter image significantly.*



*For visualization purposes only. Channel levels set does not impact image acquisition during run.

d. Check sample autofluorescence by selecting the Yellow (Autofluorescence) channel.

While the yellow channel can aid in morphology identification, for tissues with inherent low nuclear signal, the overlay may mask tissue morphology. Ensure the channel slider is fine-tuned for the sample intensity.

TIPS

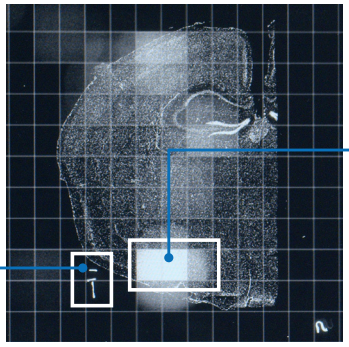
High levels of autofluorescence in overview scan is likely due to tissue morphology. Proceed with instrument run even if observed. Overview scan image is not directly comparable to data outputs.

e. Confirm sample overview scan image is free of debris.

Initiating a run with debris may further compromise image performance.

If significant debris is visible, DO NOT proceed with run. Contact support@10xgenomics.com for further instruction

Sample Overview Scan with Debris



Fixed Debris
Debris fixed on cassette carrier or slide bottom

Debris in Suspension
Floating on slide, resulting in blurry image scan. This debris will move during imaging and lead to larger artifacts

Region Selection

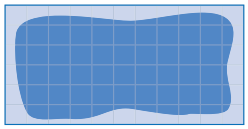
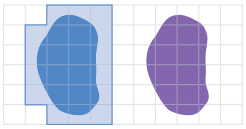
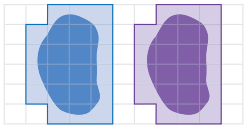
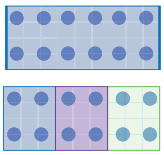
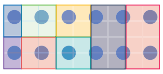
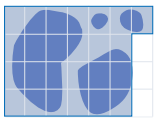
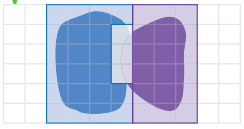
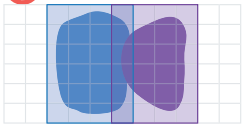
Initialize ~10-20 min › Load ~5 min › Sample Scan 1 h › **Region Sel. ~10 min** › Run 2-4 days › Cleanup ~5 min › Unload ~10 min

a. Click "Add Region" to designate imaging area(s) for each slide cassette.

TIPS *Each grid box is one field of view (FOV). FOV can only belong to one region selection and cannot be split or selected multiple times.*

*When selecting a region, **deselect all the blank FOVs**. Including blank FOVs will yield stitching errors.*

b. Follow the guidelines for region selection. (Click "?" icon on top right bar to view key instructions)

Guideline	Example
Region selection size: • Minimum: one FOV* • Maximum: entire well * one box = one FOV	Minimum size  Maximum size 
At least one region must be selected per slide	
User defines number of tissue in each region. *Sub-tissue section selection is not recommended*	
For >8 regions per slide, consolidating to one or fewer regions is recommended. ***Deselect blank FOVs.*** *A high number of regions will increase run time.*	✓ Correct  ⊘ Incorrect 
If unsure which tissue sections belong together, select all the sections as one region. ***Deselect blank FOVs.***	
FOVs in a region do not need to be contiguous.	
Overlapping regions is **not possible**. If tissue sections overlap, exclude overlapping areas from either region.	✓ Correct  ⊘ Incorrect 

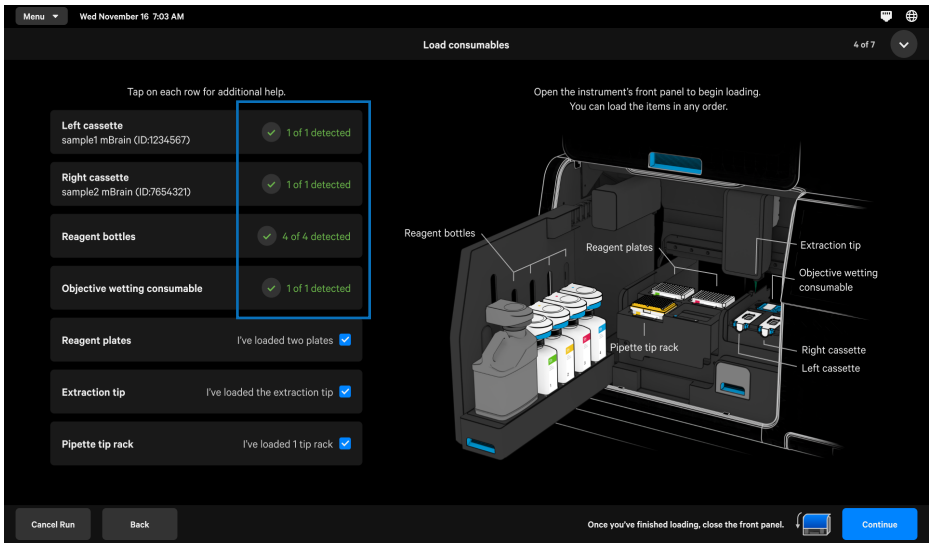
TIPS *Region names must contain only alphanumeric characters. Click pencil icon in the Regions window to edit. Region names are used to name the output directory, in the analysis summary HTML, the metrics_summary.CSV, and the experiment.xenium.*

Initiate Run

- Confirm that all consumables are loaded correctly on the touchscreen. Green check marks next to consumable indicates successful loading.
- User must manually click check box for Pipette Tip Rack and Extraction Tip to confirm before proceeding to the next step.

Consumables Detected

Green check mark indicates successful loading. User to manually verify last two consumables.



a. Confirm run settings and cassette details on the instrument screen.

b. Click "Start Run".

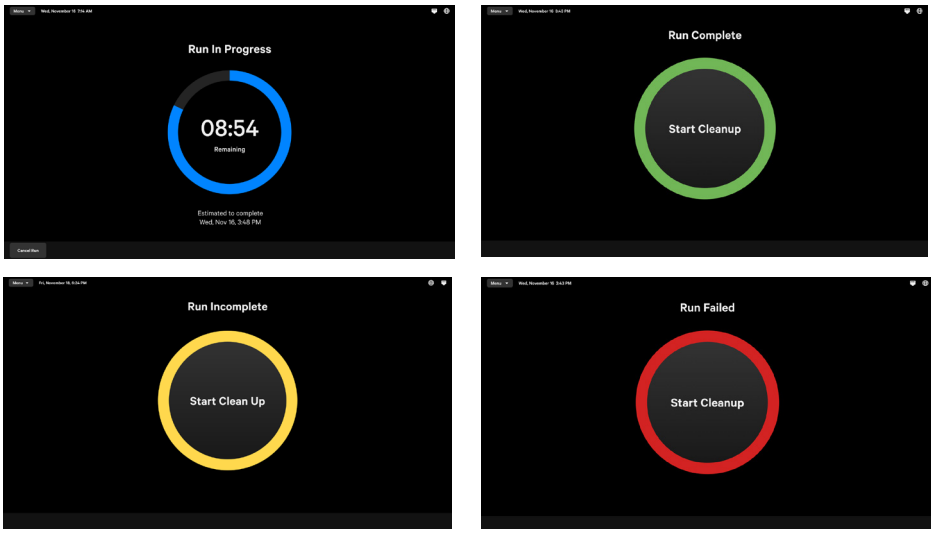
Xenium Analyzer is sensitive to vibration. Ensure sources of vibration are kept away from the instrument during the run.
DO NOT interact with the instrument during the run; avoid touching the keyboard and trackpad.

The touchscreen will display run progress and estimated time remaining.

Initiate Run
contd.

To cancel run at any time, click the "Cancel Run" button at the bottom left corner of the screen. Run information is shown in the following colors:

- **Blue** indicates run in progress.
- **Green** indicates completed run.
- **Yellow** indicates that the run is incomplete.
- **Red** indicates that the run has failed.



Refer to the Troubleshooting section for the types of errors that may be encountered when operating the Xenium Analyzer. The instrument touchscreen will guide the user through recoverable errors. If the error continues or if the instrument has seen critical errors, contact support@10xgenomics.com with the error code displayed on the screen.

Post-run Cleanup

a. After run completion, a button will appear to initiate cleaning of fluidic system. To launch, click "Start Cleanup".

Cleanup should be initiate within 72 h after a run is completed. Cleanup will stop slide hydration. Follow instructions described in the Unloading Consumables section for how to store slides following cleanup.

b. System cleanup will begin. Screen will display progress and estimated time remaining. This process should take ~5 min.

c. Click "Continue" when complete.

Unload Consumables

Refer to local and institutional guidelines for proper handling and disposal of volatile and hazardous chemicals.

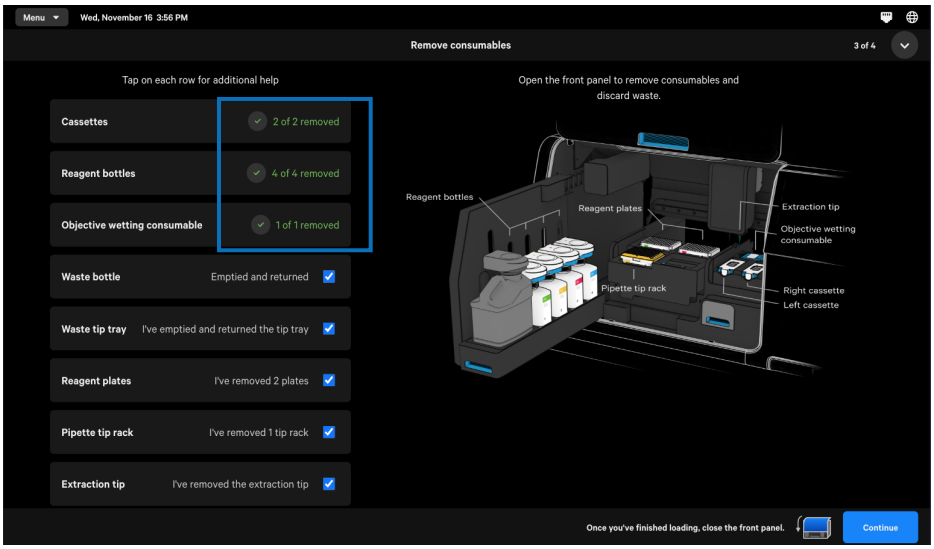
See Troubleshooting section for guidance if any errors occur during unloading consumables.

Initialize ~10-20 min > Load ~5 min > Sample Scan 1 h > Region Sel. ~10 min > Run 2-4 days > Cleanup ~5 min > **Unload** ~10 min

Open the instrument front panel, remove consumables and discard solid and liquid waste. Consumables can be unloaded in any order.

Consumables Removed

Green check mark indicates successful unloading. User to manually verify last four consumables.



Cassettes & Slides

a. Squeeze the release buttons to unlatch the cassette carrier and open the lid.

b. Remove the cassettes and clean the cassette carrier if necessary.

If liquid has leaked onto the carrier during instrument run, use a lint-free laboratory wipe with 70% isopropanol and compressed air to clean the surface of the carrier. Ensure no liquid remains to prevent it from drying onto the carrier surface.

c. Close the cassette carrier lid until it clicks into place.

d. Post-run, remove the liquid covering the slide, and add **750 µl** PBS-T to cover the sections in the cassette. Reapply the lid, and store at **4°C** for up to **1 week**.

(Optional) ***If performing post-run H&E,*** *refer to the Xenium In Situ Gene Expression - Post-Xenium Analyzer H&E Staining Demonstrated Protocol for Quencher Removal followed by H&E staining (CG000613).*

Unload Consumables *contd.*

Waste Bottle *(Reusable)*

a. Slide the bottle carrier tray out on the left side of the instrument.

b. Remove the liquid Waste Bottle and discard the liquid. Each run will generate ~300-500 ml liquid waste.

The waste includes potentially volatile and hazardous chemicals. Follow institution or local guidelines for proper waste disposal.

c. Place the empty bottle back in first position of bottle carrier.

Reagent Bottles

a. Squeeze the bottle carrier caps and raise them until they reach a height tall enough to remove bottles.

b. Remove bottles from carrier. Uncap the reagent bottle cap and empty the used reagent bottles at the appropriate liquid waste disposal following institution or local guidelines. Discard bottles when done.

c. Push the bottle carrier back into place.

Objective Wetting Consumable

a. Remove the Objective Wetting Consumable from behind the cassette carrier.

b. Discard the used Objective Wetting Consumable following institution or local guidelines for proper waste disposal.

Reagent Plates

a. Remove the used reagent plates from the plate deck.

b. Discard the used reagent plates following institution or local guidelines for proper waste disposal.

Condensation under reagent plates post-run may be visible and is normal. Following plate removal, dry the area if condensation has occured.

Pipette Tip Rack

a. Remove the tip rack from the plate deck.

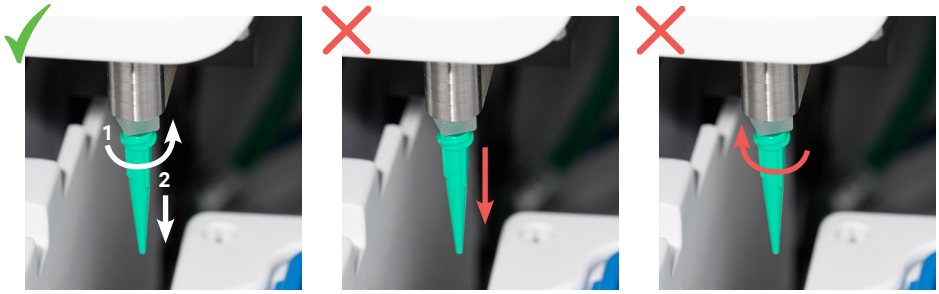
b. Discard tips following institution or local guidelines for proper waste disposal.

Unload Consumables *contd.*

Extraction Tip

a. Grip the top part of the Extraction Tip, twist it slightly in counterclockwise direction and then pull the tip down to remove.

Removing the Extraction Tip



Grip top part of tip, twist counterclockwise and then pull down.

DO NOT pull down directly without twisting counterclockwise.

DO NOT twist clockwise.

b. Discard the Extraction Tip following institution or local guidelines for proper waste disposal.

Waste Tip Tray *(Reusable)*

a. Slide the waste tip drawer open and remove the Waste Tip Tray.

b. Discard the used pipette tips following institution or local guidelines for proper waste disposal.

c. Place the empty Waste Tip Tray into the waste tip drawer and close.

Once all consumables are removed or emptied, close the instrument front panel and click “Continue”.

Powering OFF Instrument

While instrument shutdown is not required, user may power off instrument if it is expected to be idle for long periods of time.

a. Retrieve data from instrument following completed run (Refer to Data Output for instructions)

b. Press the blue power button for >3 sec located on the side panel (right)

Do NOT switch off power buttons at the rear of the instrument. They must remain on for proper function.

Do NOT use the Touchscreen to shutdown instrument

10x GENOMICS

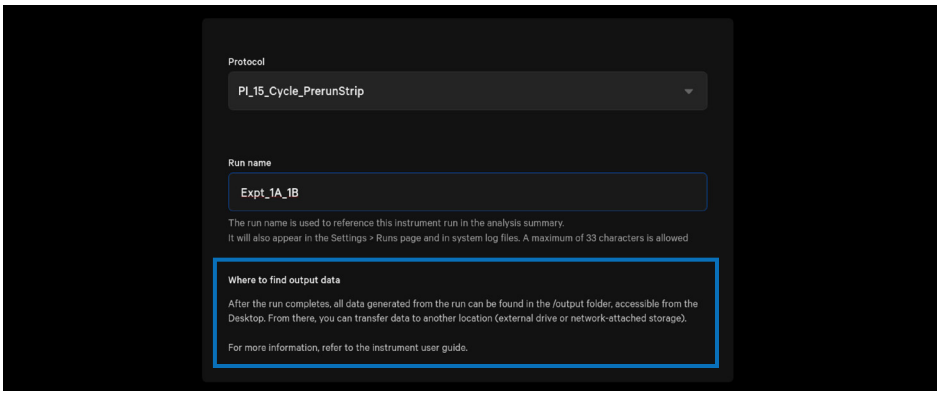
Data Output

Data Output

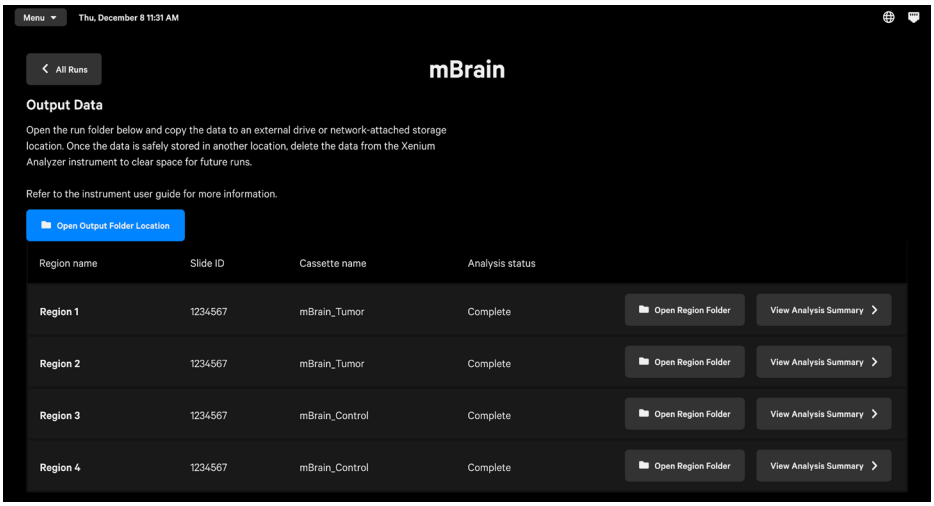
During every Xenium Analyzer run, image processing, decoding, and secondary analysis are performed real time on-instrument, generating a run-specific data output folder.

Data Output Location

The output data location and transfer instructions are available on the instrument screen during run setup and after the run completes.



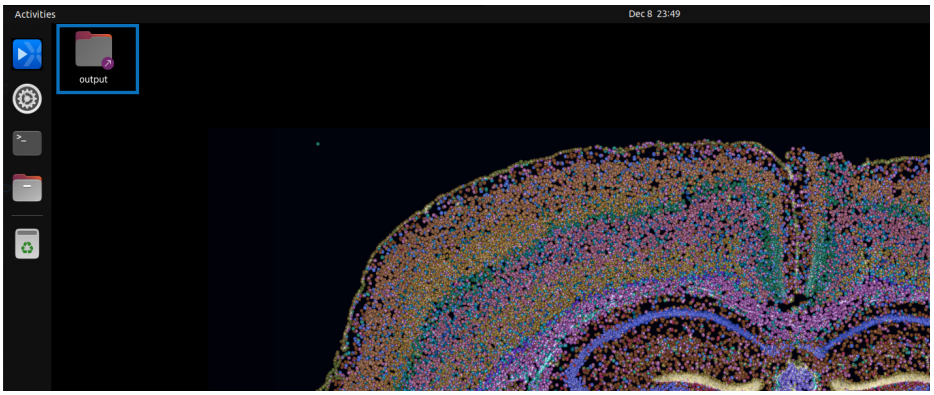
After the run is complete, data generated across all the runs can be accessed under “Menu > Open Settings > Runs”.



Click “Open Output Folder Location” to access the top-level output folder on the desktop. Click the individual runs to open a run-specific screen. To access the region-specific output folder, click “Open Region Folder”. A summary of the analysis is available in “View Analysis Summary” folder.

Data Output
contd.

All run data will be stored in the output directory on the Xenium Analysis Computer and can be accessed through the output folder of the desktop.



For more information about the output files within each subfolder, refer to the 10x Genomics Support website.

Data Storage Capacity

The Xenium Analysis Computer has a storage capacity of 8 TB NVMe. This capacity is adequate for storing data acquired from more than 50 Xenium Analyzer runs, assuming that the data is acquired across the full imaging area of two Xenium slides for hundreds of RNA targets.

Data Export

Exporting the output data after each instrument run is highly recommended to reduce the system load and to avoid any possibility of losing run data. User will be responsible for managing and deleting output bundles from the runs.

Export data only after the run in complete and not while the run is in progress. DO NOT interact with the instrument during the run; avoid touching the keyboard and the trackpad.

Data Output
contd.

Multiple options are available to export run data from the instrument.

Local Area Network (LAN) (Recommended Option)

- Users can also work with their institution's IT department to set up Local Area Network (LAN) for data transfers. Xenium Analyzer can be configured to work with non-persistent networks such as Network File Share (NFS) or Common Internet File System (CIFS).

Portable USB drive (Alternative Option)

- Attach a portable USB drive to the USB port on the Xenium Analysis Computer to export the output files.
- USB drive must have ≥ 256 GB storage capacity, ≥ version 3.0, and be pre-formatted to the exFAT file system, which is compatible with the operating systems indicated below.

Label cannot contain spaces or the following characters !@#$%&)+= Failure to comply will cause user to be unable to write to the drive.*

File System	Windows *(7/8/10)*	macOS *(10.6.5 & later)*	Ubuntu Linux
exFAT	Yes	Yes	Yes

- Once copy shows complete, user should eject USB properly (do not pulling from system until eject is successful). If eject operation fails indicating the device is busy, wait until that is resolved and eject is successful before removing USB.
- Persistent network mounts can also be configured during instrument installation or later. For more information, contact support@10xgenomics.com.

10x GENOMICS

Maintenance

Maintenance

Cleanup After Run

After run completion, a button will appear on the instrument touchscreen to initiate cleaning of the instrument fluidic system. The screen will display progress and the estimated time remaining. This process will take **~5 min**.

Interior

Wipe the instrument deck with 70% ethanol or 70% isopropanol, including the fluidic line inlets and outlets (reagent buffer bottle inlets, waste bottle outlet, extraction tip inlet). Use compressed air to dry and remove debris as needed.

DO NOT use 5-10% bleach for routine cleaning. In very rare instances that require decontamination as per an institution's protocol (for example moving from a BSL2 facility), 5-10% bleach solution may be used for wiping the deck. Frequency of such cleaning should not exceed 1-2 times during the life of the instrument.

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

Exterior

The exterior of the Xenium Analyzer 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 damp lab towel.

Cassette Carrier (Inside)

Always clean carriers prior to loading a run. If liquid has leaked during a run, clean carriers after a run when unloading.

a. Spray 70% isopropanol onto a lint-free laboratory wipe and clean the surface of the carrier, paying attention to the raised areas that come into contact with the slide. Let evaporate.

   i. Optional: Spray 70% isopropanol on a cotton swab and use to clean off crevices if necessary.

b. Use compressed air to remove any remaining lint paying close attention to raised areas. Confirm surface is dry.

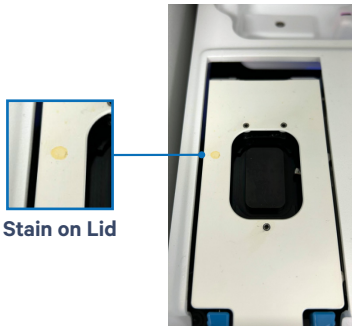
A dry, clean, lint-free surface on both the slide bottom and instrument cassette carrier is critical for a proper instrument run. Any debris or lint can interfere with image acquisition.

Maintenance
contd.

Cassette Carrier (Lid)

In some cases, if imaging buffer comes into contact with the lid of the cassette carrier, a stain can occur and will be visible after the run has completed. This stain is cosmetic and has no impact on instrument performance. However, cleaning following a run is advised as older stains are typically more difficult to remove.

Stain on Cassette Carrier



For relatively new stains (< 1 week), gently wipe the stain with 100% acetone with a lint-free wipe.

For older stains that cannot be removed using acetone (> 1 week), gently wipe the stain with 1M NaOH with a lint-free wipe. If necessary, a soft bristle brush may be used to aid in removal.

Refer to SDSs for handling guidance and safety practices (such as PPE). Dispose of any waste following regional and institutional guidelines.

Only use the solvents recommended above at the specified concentration or molarity. DO NOT use alternative solvents to remove stain as they can damage the coating on the cassette carrier. DO NOT use solvents to clean any other surfaces on the instrument.

Maintenance
contd.

Powering OFF instrument

While instrument shutdown is not required, user may power off instrument if it is expected to be idle for long periods of time.

To power off the instrument, press and hold the power button on the right side of the instrument for **>3 sec**.

Do NOT turn off the Breaker Switch or Main Power Switch. Both must remain in the ON position for proper instrument function.

Do NOT use the Touchscreen to shutdown the instrument.

Service

For routine preventative maintenance, 10x Genomics will contact the user at regular intervals to schedule and perform routine service and maintenance.

Electrical shock hazard. DO NOT open the Xenium Analyzer 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 Xenium Analyzer 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). For more information, contact support@10xgenomics.com.

Only the power cords supplied with the Xenium Analyzer will be used during installation. DO NOT replace cords with a non-approved power cord as it may be inadequately rated to handle the electrical loads.

Maintenance
contd.

Environmental Requirements

It is the design intent of the Xenium Analyzer that it be used in a typical indoor laboratory environment. The instrument's operating temperature is 19–25°C (66–77°F), humidity 80% Max (Non-Condensing).
See Instrument Specifications.

Troubleshooting

Troubleshooting

Check Assembled Cassette for Leaks

Prior to loading the assembled cassette on the instrument, check that no liquid is leaking from the assembly as this can negatively impact assay performance. Dry the front and the back of the slide completely using a lint-free laboratory wipe while avoiding touching or damaging the tissue sections. Inspect the slide carefully to ensure it is seated fully within the cassette before assembly.

Scenarios that may indicate improper Xenium Cassette assembly include:

- Cassette does not click shut or appears domed/has a gap after assembly (see image below).
- Assembly is placed on a dry surface, the surface is wet following removal of the assembly, indicating reagent leakage from the cassette.

If cassette assembly is leaking prior to instrument run, disassemble and reassemble the cassette as instructed below. Add **1,000 µl** PBS-T to cover the slide before loading onto the instrument. **Confirm cassette is no longer leaking before loading.** Leaks may cause instrument damage.

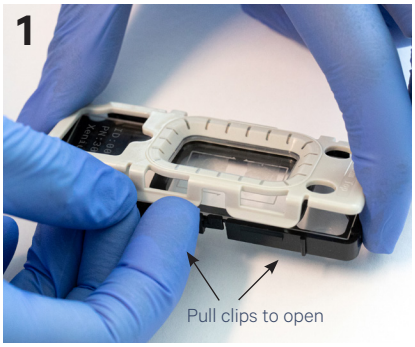
Ensure the slide sections do not dry out during the process.

Disassemble Xenium Cassette

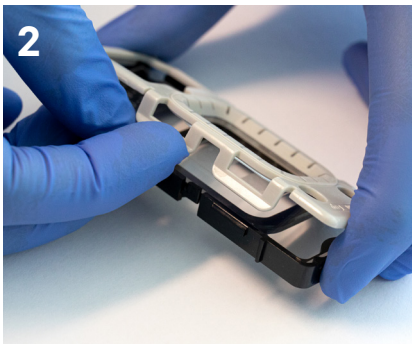
Exercise caution when handling slide edges to prevent injury.

Cassette assembly with a gap will result in reagent leakage

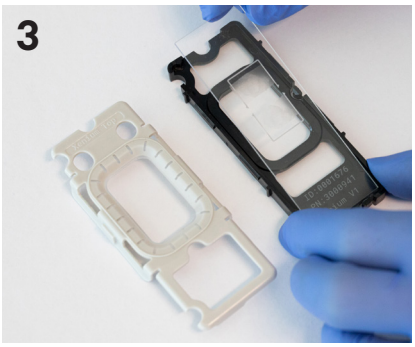
Pull inner clips from inner tabs to detach top and bottom halves of cassette



Open cassette by continuing to lift inner clips upward



Hold slide by the label and lift slide out from bottom half



Slides in images are representative.

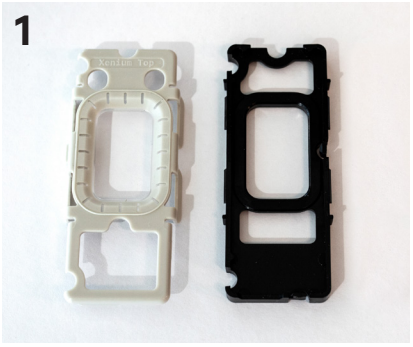
Troubleshooting
contd.

Exercise caution when handling slide edges to prevent injury.

Slides in images are representative.

Reassemble Xenium Cassette

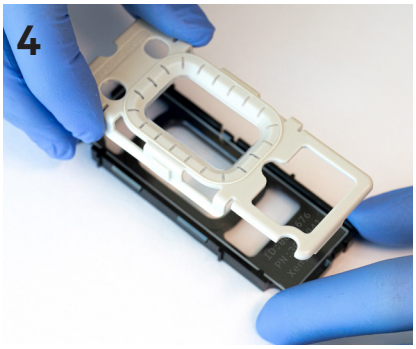
Place top and bottom halves of cassette on bench



Place Xenium slide with tissue side facing upwards into bottom half of cassette; ensure label is toward bottom of cassette

Press slide down into grooves of the bottom half of the cassette until it sits firmly in place

Secure clips of top half with tabs of bottom half (on both sides)



Apply even pressure on top of cassette until all clips click shut. Verify that clips are completely secured over tabs

Add 1,000 µl PBS-T to cover the slide before loading onto the instrument

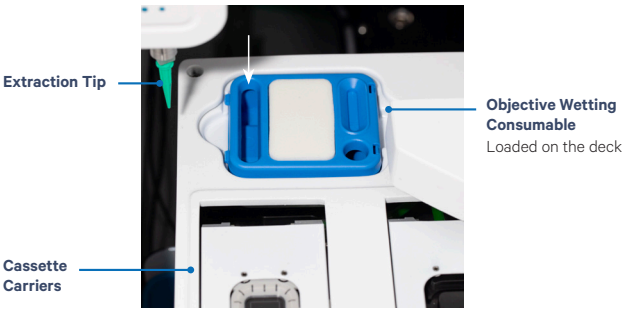
Troubleshooting
contd.

Full Instrument Shutdown

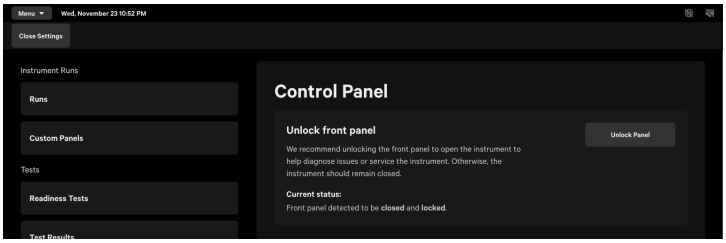
In some instances, complete instrument power shutdown and power on is required. Please only do so when instructed by 10x Genomics personnel. Contact support@10xgenomics.com for assistance.

Errors while Loading and Unloading Consumables

Listed below are errors (along with solutions) that may occur when loading and unloading consumables on the instrument and during data analysis.

Error	Solution
During loading and unloading consumables	
Objective Wetting Consumable not present or not full	Place a new, unused objective wetting consumable in the correct location on the instrument deck (behind the cassette carrier; the reagent priming reservoir should be on the left (white arrow). 
Instrument front panel is not closed and/or locked	Ensure that the front panel is securely closed and try again.
Check bottle carrier	Ensure that the bottle carrier with reagent bottles and Waste Bottle is pushed all the way into position inside the instrument.
Missing Waste Tip Tray	Slide out the waste tip drawer and place the empty Waste Tip Tray inside the drawer. Close the drawer and proceed.
Empty Waste Tip Tray	Slide out the waste tip drawer and remove the tip tray. Discard used pipette tips and place the empty tip tray inside the drawer. Close the drawer and proceed.
Left or right cassette is missing	Load an assembled cassette in the correct position on the cassette carrier.
Cassette Carrier lid not properly closed	Ensure that both cassette carrier lids click into place to be properly closed.
Missing Waste Bottle	Place the Waste Bottle in the bottle carrier. Push the bottle carrier caps down to the top of the Waste Bottle.
Empty the Waste Bottle	Remove the Waste Bottle from the carrier and discard the waste. Follow institutional or local guidelines for proper waste disposal. Return the bottle to the bottle carrier. Slide the bottle carrier back and proceed to the next step.

Troubleshooting
contd.

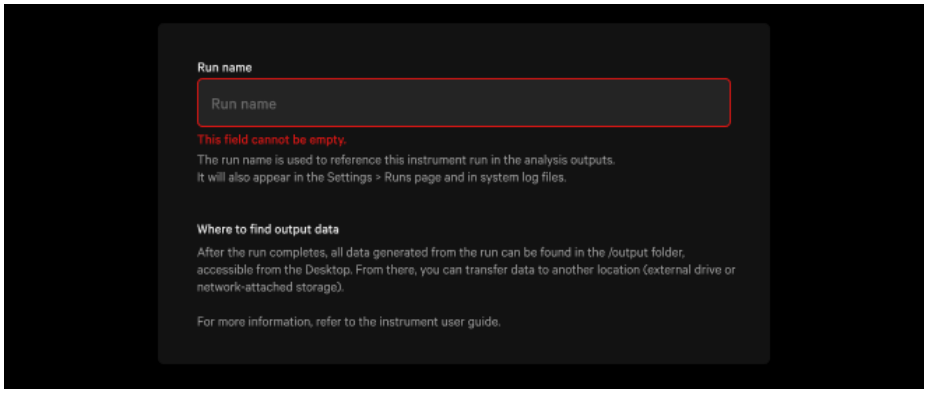
Error	Solution
During loading and unloading consumables	
Need to open the front panel to access the instrument deck prior to starting a run	Unlock the front panel from Menu Settings, accessible on the top bar of the screen. 
During data analysis	
Insufficient storage available	There is insufficient storage to save analysis output data. Delete data from previous runs from the output directory. Contact support@10xgenomics.com for assistance.
During data analysis *may be recoverable with assistance from 10x Support team*	
Analysis failed to start	A problem prevents starting the analysis. The run has been terminated. Samples will be kept hydrated until run cleanup. Refer to the Unloading Consumables section for guidance regarding keeping samples stable after unloading. Contact support@10xgenomics.com for assistance.
Analysis failed	A problem has occurred during analysis. The run has been terminated. Samples will be kept hydrated until run cleanup. Refer to the Unloading Consumables section for guidance regarding keeping samples stable after unloading. Contact support@10xgenomics.com for assistance.
Region analysis failed to finalize	An error occurred during analysis for a specific region “{{Region}}”. Analysis will continue for the other regions. After the run is complete, contact support@10xgenomics.com for assistance.
Failed to generate output data files	Analysis output run data for the region “{{Region}}” could not be saved. Saving output for the other regions will continue. After the run is complete, contact support@10xgenomics.com for assistance.
Cannot save output data	There was a problem in saving analysis output data. Contact support@10xgenomics.com for assistance.
Other	
Screen/instrument frozen	Press and hold the power button on right side panel for >3 sec until instrument is turned off. Wait for at least 3 minutes and then power on the instrument by pressing and holding the power button for >3 sec until instrument turns on. Wait for at least 3 minutes to allow all systems to power on fully. Contact support@10xgenomics.com for assistance if issue persists.

Errors

Listed below are the types of errors that may be encountered when operating the Xenium Analyzer. The instrument touchscreen will guide the user through recoverable errors. If the error continues, or if the instrument has seen critical errors, contact support@10xgenomics.com with the error code displayed on the screen.

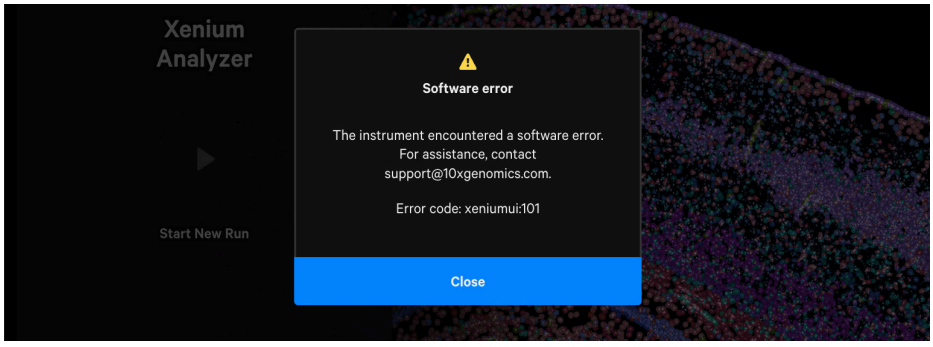
Contextual Error Messages

While completing information fields in the instrument screen, invalid input may be detected. The field where input correction is needed will be highlighted in a red bounding box and guidance on resolving the error will appear adjacent to the input field.



Error Alerts

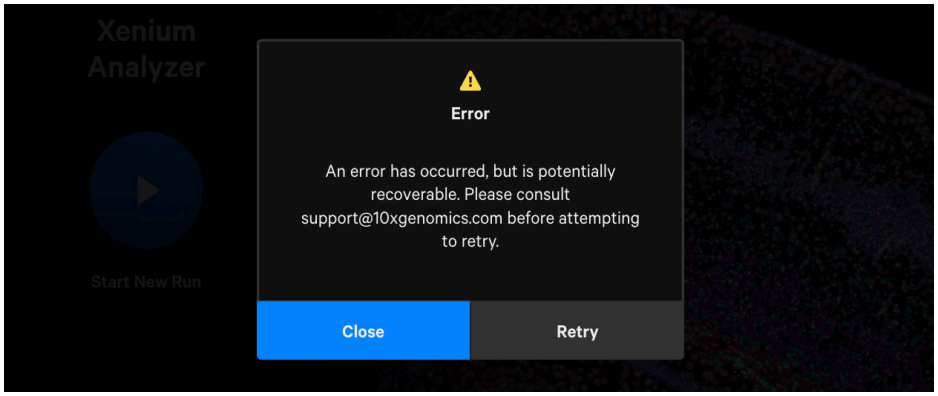
When an error occurs outside of completing information fields, an error alert will appear on the screen. Follow the instructions displayed on the screen.



Errors
contd.

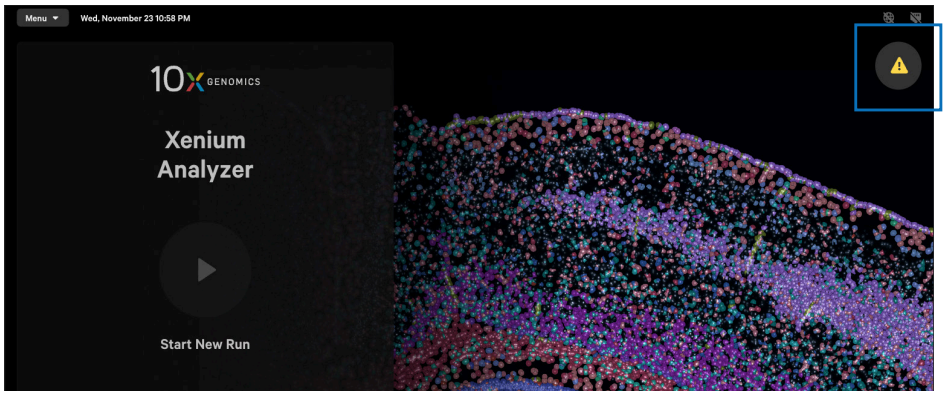
System Retry

Some errors may provide the option to retry the previous system operation. It is recommended to email support@10xgenomics.com for assistance before attempting retries.



Home Screen Error Indicator

Some errors may prevent the user from starting a new run. An error button will appear in the upper right corner of the home screen. Click the button to open the error alert and follow the instructions displayed on the screen.



Critical Errors

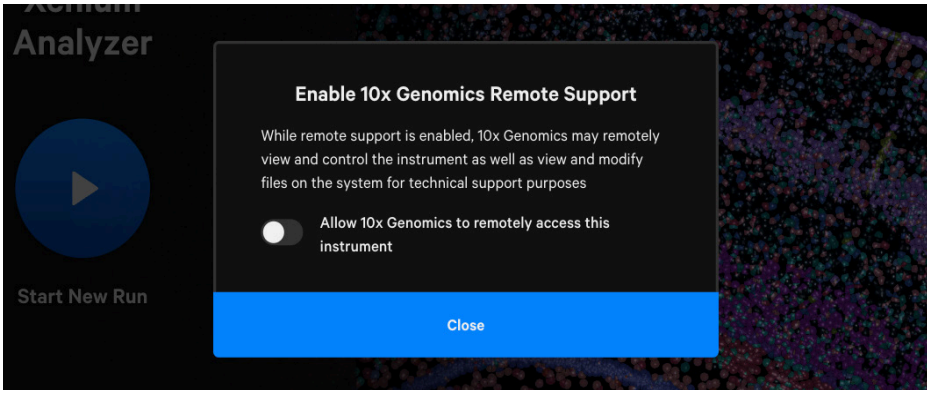
Contact support@10xgenomics.com with the error code. Do not proceed with any further runs.

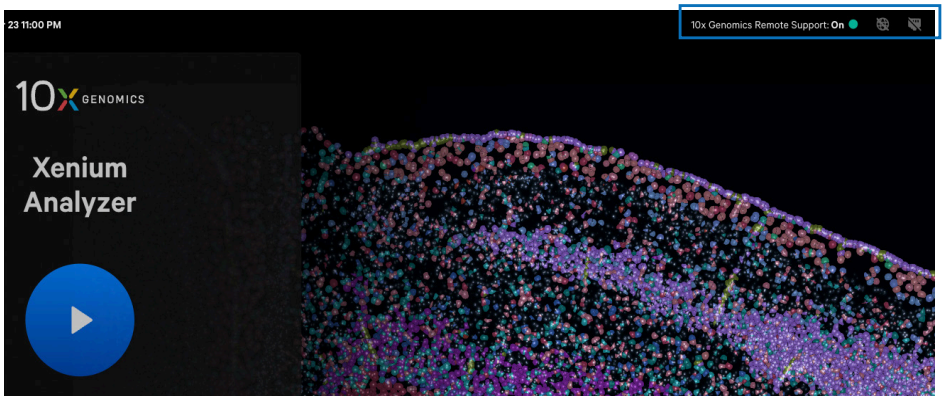
Errors
contd.

Enable Remote Support for Troubleshooting Guidance

When contacting 10x Genomics for technical support, 10x Genomics personnel may remotely access the instrument for providing troubleshooting guidance. Enable remote support by clicking "Menu > Remote Support", and then moving the toggle to ON. Once enabled, the header bar on the instrument screen will display "10x Genomics Remote Support On."

Authorized 10x Genomics personnel will remotely access Xenium Analyzer instruments when given explicit permission from the user to do so. Only data necessary to provide troubleshooting support will be recovered and handled.





Errors
contd.

Remote access may also be enabled while a run is not in progress from the Connectivity Settings, found by navigating to "Menu > Connectivity".