10x GENOMICS

User Guide | CG000584 | Rev K

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 K

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 K

Title

Xenium Analyzer User Guide

Revision

Rev J to Rev K

Revision Date

August 2025

Description of Changes

- Added information regarding Xenium Gene and Protein Expression Workflow (pg 7)
- Updated electrical requirements in specifications table (pg 9)
- Added Xenium Decoding Reagent Module A (Universal) to all related sections (pgs 32, 43-47, 51, 54-58, 61)
- Added new Xenium Gene and Protein Expression Reagent Preparation and Loading chapter (pgs 63-73)
- Updated System Operation to reflect updates related to XOA 4.0 release (75-77, 79)
- Refined language regarding debris in Sample Scanning section (pg 86)
- Updated for general 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. The Xenium Analyzer instrument seamlessly integrates upstream assay workflows, high-resolution imaging, decoding, and onboard data analysis.

- Intuitive instrument design and interface.
- Automated in situ platform that performs successive rounds of fluorescent probe hybridization, imaging, and probe removal to generate an optical signature for each gene transcript.
- Image processing, decoding, and secondary analysis are performed in real-time on-instrument.
- Data visualization with the Xenium Explorer desktop software and data reanalysis with the Xenium Ranger Linux software.

Assay Workflows Compatible with the Xenium Analyzer Instrument

Xenium Workflow
Xenium v1 Gene Expression
• Xenium In Situ Gene Expression (CG000582) • Xenium In Situ Gene Expression with optional Cell Segmentation Staining (CG000749)
Xenium Prime Gene Expression
• Xenium Prime In Situ Gene Expression with optional Cell Segmentation Staining (CG000760)
Xenium Gene and Protein Expression
• Xenium In Situ Gene and Protein Expression with Cell Segmentation Staining User Guide (CG000819)

Reagent preparation and loading differ based on the workflow performed. Failure to follow carefully can affect output data. In this user guide, reagent preparation and loading are organized by workflow and noted by colored tabs on the right side of the page.

Ensure the appropriate workflow is selected and followed.

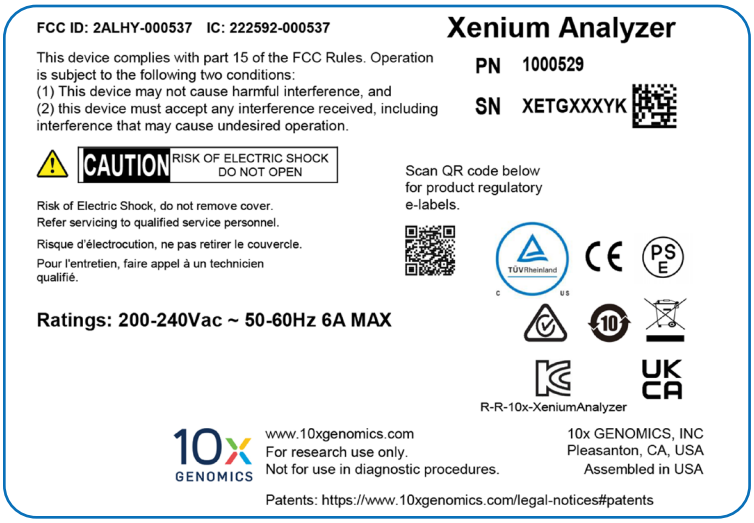
Xenium v1 Gene Expression workflows

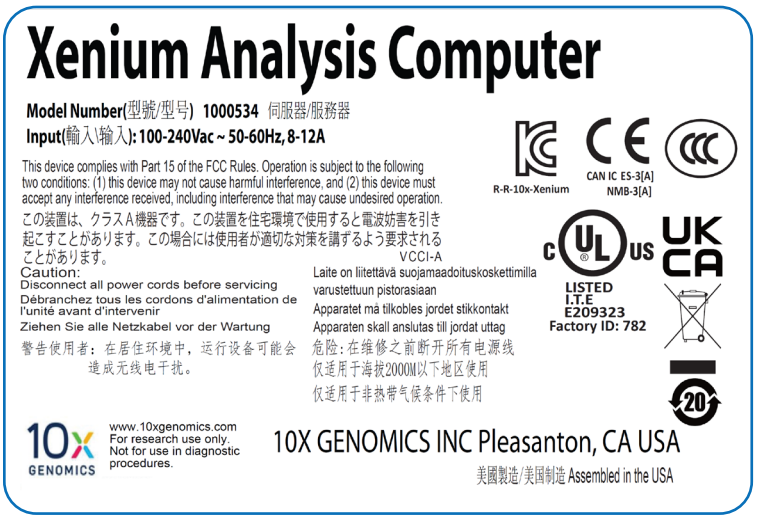
Xenium Prime Gene Expression workflow

Xenium Gene and Protein Expression workflow

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: ~1,182 lb (536.1 kg)*
Xenium Analysis Computer	~57 lb/25.8 kg		
Vibration Isolation Table	~575 lb/260.8 kg		
Dimensions	**W**	**D**	**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	31.1”/79.0 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	800W, 200–240 VAC, 50–60Hz, 4.0A–3.3A*		
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, noncondensing		
Altitude	Altitude up to 2,000 m (1.2 miles) 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 Ethernet Link Speed: 10Gbps Operating System: Ubuntu 22.04 LTS (nonconfigurable		
Xenium Analysis Computer Electrical Specifications	1200W, 200–240 VAC, 50–60Hz, 6.0A–5.0A*		
Vibration Isolation Table Gauge Specifications**	Source air supply (compressor, wall air, tank, etc.): ~80–150 psi Table air supply: ~70–80 psi Table leg pressure: ~50–60 psi		

**Electrical requirements dependent on region/country*

***Contact support@10xgenomics.com if table specifications are out of range.*

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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: 1,183 lb (536.1 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 adapters. Using any other cables and adapters 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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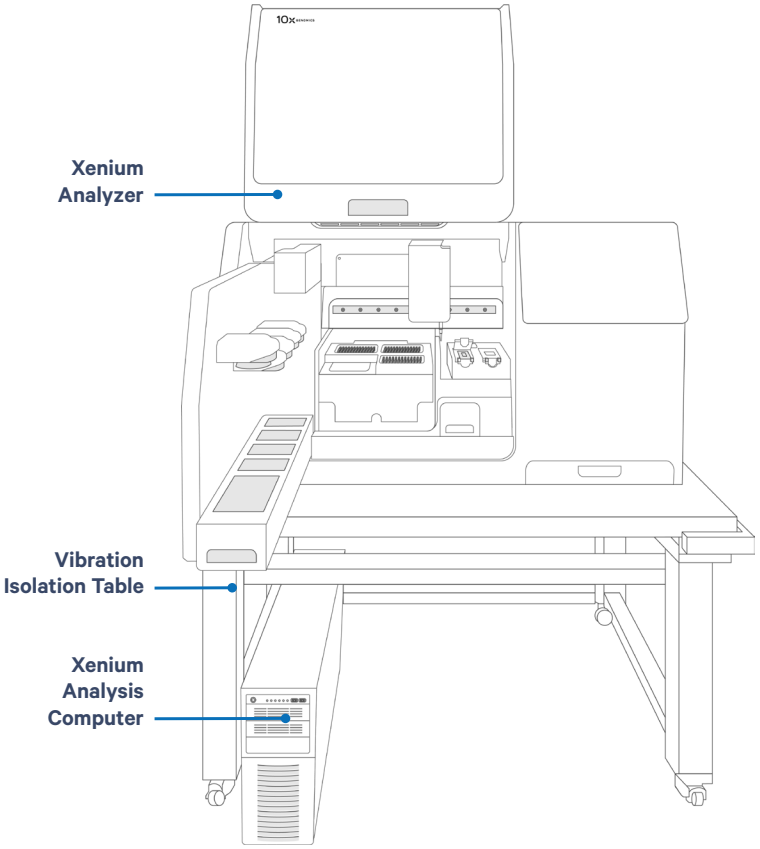
System Components

Instrument Installation

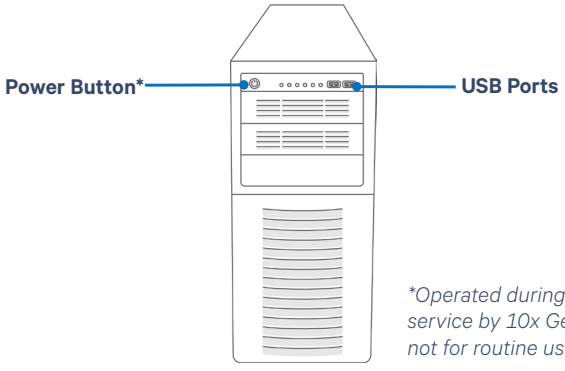
10x Genomics will provide complete installation services necessary for Xenium Analyzer, Vibration Isolation Table, and Xenium Analysis Computer.

Full Instrument Setup



System Components - *Xenium Analysis Computer*

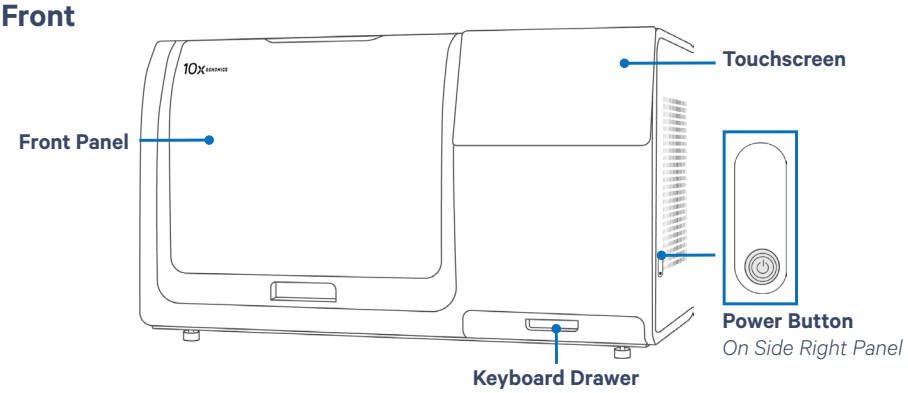
Front



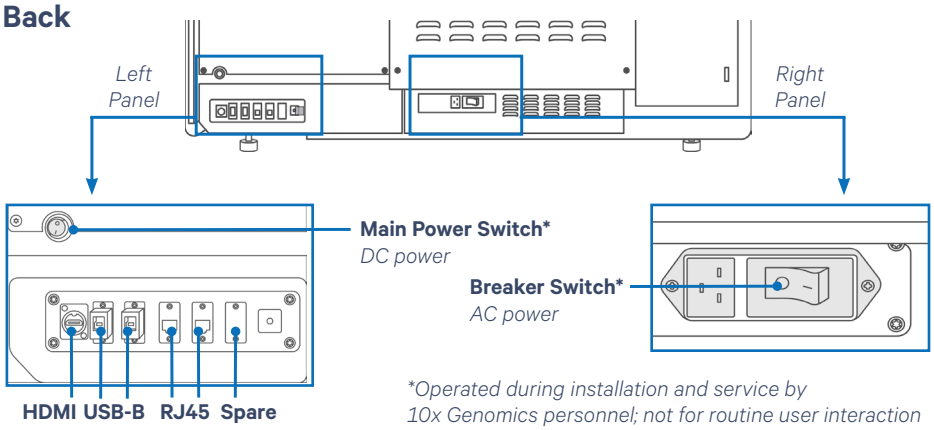
**Operated during installation and service by 10x Genomics personnel; not for routine user interaction*

System Components - *Xenium Analyzer*

Front

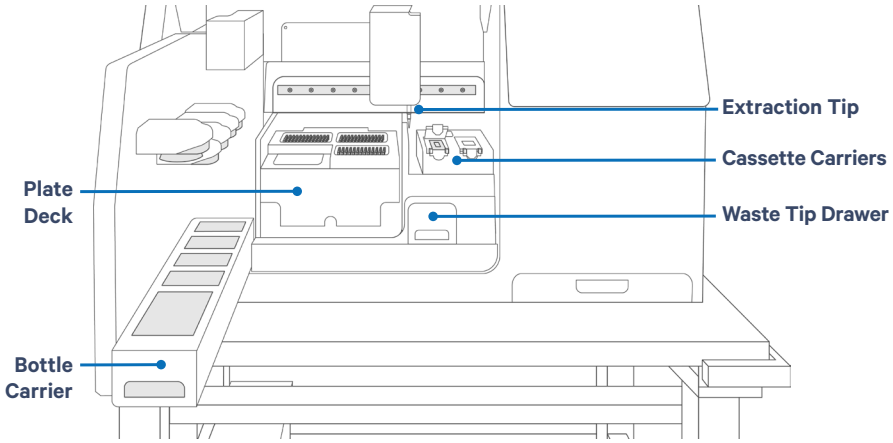


Back

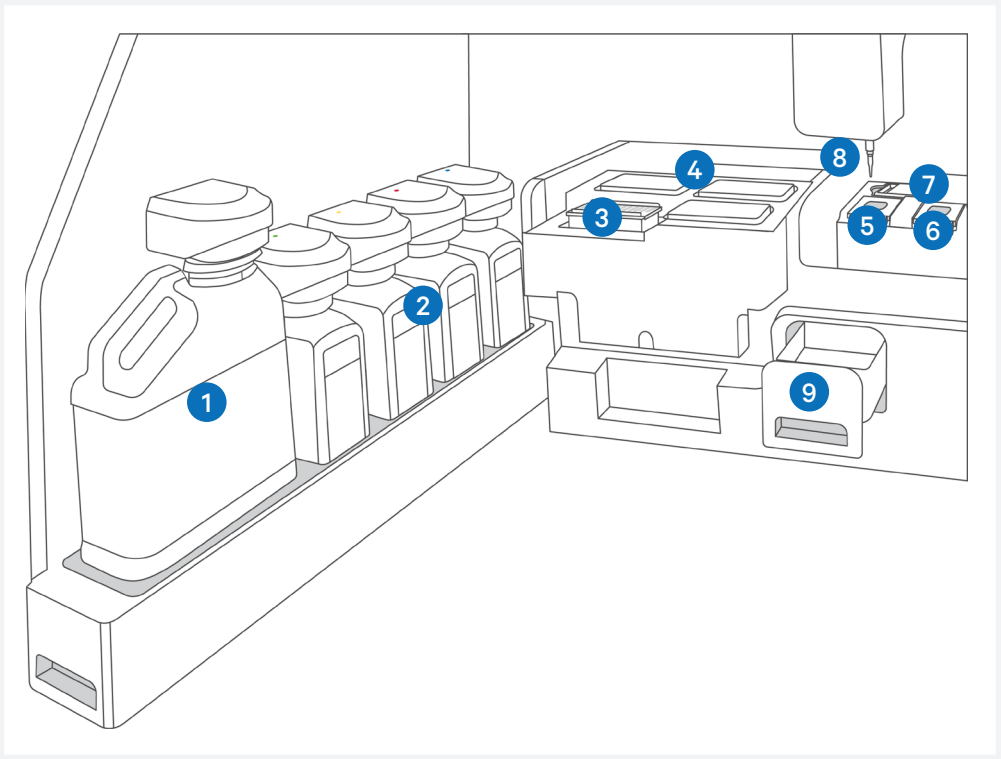


*Operated during installation and service by 10x Genomics personnel; not for routine user interaction

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 or 3, depending on assay method)*
Cassette Carrier	5	Left Cassette *(lid open)*
	6	Right Cassette *(lid closed)*
	7	Objective Wetting Consumable (OWC)
	8	Extraction Tip
	9	Waste Tip Drawer *(Waste tip tray inside)*

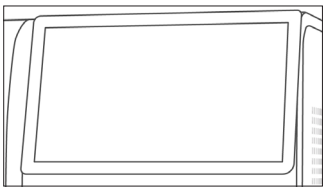
Hardware Components - *Xenium Analyzer*

The Xenium Analyzer includes the following hardware components. See the System Components and Deck Layout sections for the specific location of each hardware component.

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

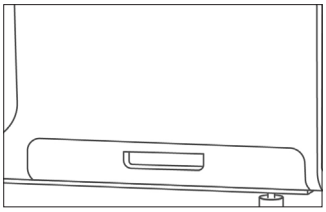
Touchscreen

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



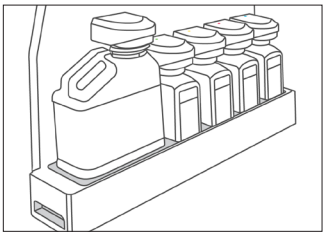
Keyboard Drawer

The wireless keyboard drawer is located on the bottom-right corner of the instrument, underneath the touchscreen. A keyboard with trackpad is provided.



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. Waste Tip Tray inside holds solid waste generated by run.

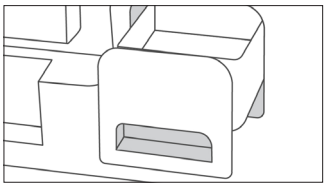
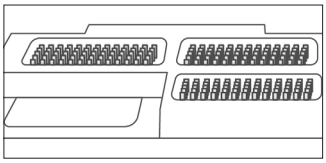


Plate Deck

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

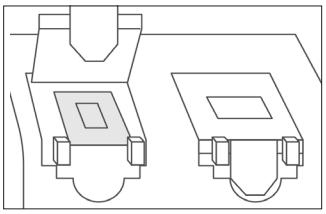


Cassette Carrier

Two cassette carriers sit to the right of the plate deck. Front tabs are used to open carriers to load slide cassettes.

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 crush/break slide.

DO NOT proceed with instrument run if slide is cracked or broken. Cracked or broken slides will result in instrument failure and replacement reagents will not be provided.



Caution: *Hot surface (gray region) during instrument run.*

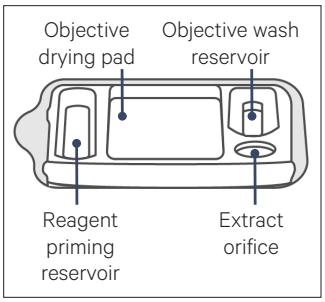
Accessories & Consumables

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. See the Deck Layout section for specific locations of each item.

Not all accessories and consumables are shipped with the instrument. See the Accessory Kits and Reagent Kit & Consumables sections for complete details.

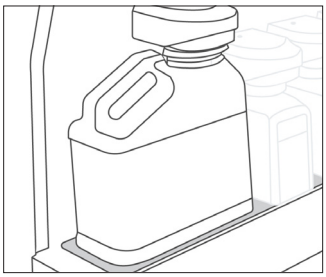
Objective Wetting Consumable (OWC)

The OWC 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. **Single use only**. Discard after each run.



Waste Bottle *(Reusable)*

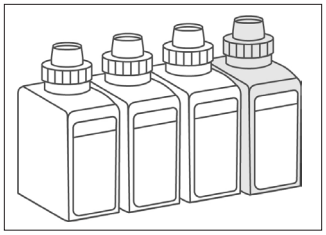
The liquid Waste Bottle sits in the front position of the bottle carrier and collects liquid waste generated during run. Waste Bottle is reusable. Empty after each run and return back to position before starting the next run.



Follow institutional or local guidelines for proper liquid waste disposal.

Reagent Bottles (x4)

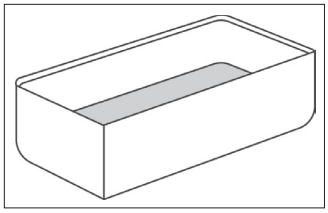
Four reagent bottles sit in the bottle carrier behind the Waste Bottle. Bottles are color-coded to the instrument. Place bottles in the correct order when prompted to.



Follow institutional or local guidelines for proper liquid waste disposal.

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.

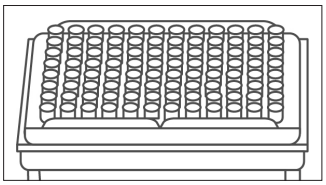


Follow institutional or local guidelines for proper solid waste disposal.

Accessories & Consumables
contd.

Pipette Tip Rack

Pipette tip rack sit in the front left area of the plate deck and is labeled with a T on the front. **Single use only**. Discard after each run.

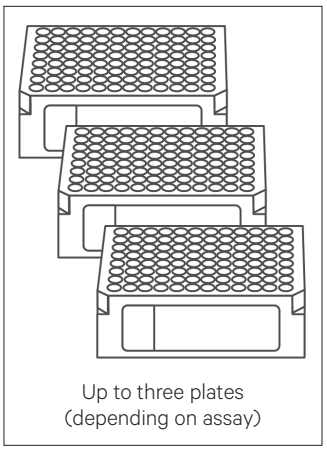


Reagent Plates

Two required reagent plates (Plate A and B) sit behind the Pipette Tip Rack. A third reagent plate sits right of the Pipette Tip Rack and is required depending on assay method.

Plates are specific to its location and contains unique reagents. Specific handling and preparation are required prior to loading and are described in the Reagent Plate Preparation section. **Single use only**. Discard after each run.

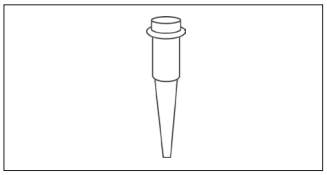
The foil seal on the plates should be intact when loading instrument. DO NOT use plates if foil seal is punctured.



Up to three plates (depending on assay)

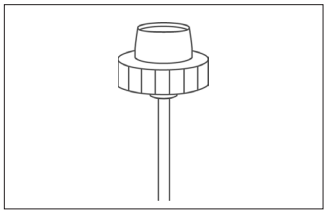
Extraction Tip

The Extraction Tip transfers liquid during run **Single use only**. Discard 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. **Single use only**. Discard after each run.



Instrument Bundle and Accessory Kits

Xenium Instrument Bundle, PN-1000569

Includes Xenium Analyzer and Xenium Analysis Computer* (PN-1000529)
Instrument Accessory Kit Module A & Module B
Thermocycler Adaptor & Thermocycler Adaptor v2**

**Xenium Analysis Computer only PN-1000534*
***Required for sample preparation performed prior to instrument loading (CG000578, CG000579). Adaptor version depends on Xenium assay workflow performed.*

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

Item	#	Part Number
Waste Bottle	1	3000955
Xenium Waste Tip Tray	1	3000957

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

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
USB 3.2, 1TB, USB-A & USB-C	1	3002174
MDP to HDMI Adaptor	1	3002655

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

Xenium Thermocycler Adaptor, PN-1000623 *shipped with instrument*

Item	#	Part Number
Xenium Thermocycler Adaptor	1	3000954

Xenium Thermocycler Adaptor v2, PN-1000739 *shipped with instrument*

Item	#	Part Number
Xenium Thermocycler Adaptor v2*	1	3002207

Gene Panels
(for all workflows)

Prior to executing the Xenium In Situ Gene Expression workflow, ensure a compatible gene panel has been selected. 10x Genomics provides the following types of gene 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

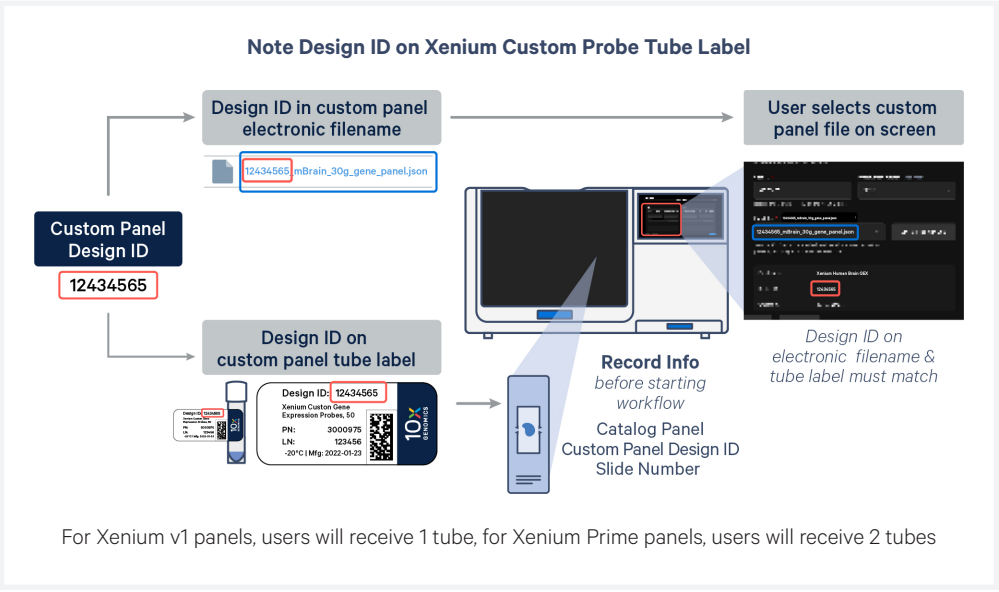
Visit the 10x Genomics Support website for the most updated information regarding all the available pre-designed panels.

Compatible Custom Gene Panels

Contact your 10x Genomics Sales Executive regarding custom gene panels designs compatible with pre-designed or standalone custom gene panels. If you do not know your Sales Executive, contact customerservice@10xgenomics.com.

Lead time for acquiring custom panels is up to 8 weeks (1-4 weeks for design, 4 weeks for manufacturing). Visit the 10x Genomics website for the most updated information.

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



For Xenium v1 panels, users will receive 1 tube, for Xenium Prime panels, users will receive 2 tubes

Protein Subpanels

(for Xenium In Situ Gene and Protein Expression workflow)

10x Genomics offers protein subpanels related to immunology, cancer, and proliferation markers. Select one or more prior to performing the Xenium In Situ Gene and Protein Expression workflow.

Xenium Protein subpanels are compatible with human FFPE tissues and must be used in conjunction with a human Xenium v1 pre-designed or custom gene panel.

Consult the 10x Genomics website for detailed information regarding protein subpanel overlap with pre-designed gene panels.

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. Consult 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 Milli-Q 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 *(concentration 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. Consult 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			
☐	70% Ethanol			
☐	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 image representing the tissue section. The pipeline detects puncta in every cycle and every image to observe all potential mRNA. These puncta are decoded into gene IDs, and each decoded transcript is assigned a quality score.

If samples were prepared using the Multi-Tissue Stain Mix, multimodal cell segmentation is performed to define cell boundaries and assign transcripts to cells. Otherwise, nuclear expansion is performed using the DAPI images. Finally, the pipeline outputs a bundle of data files (see Data Output) that can be exported for further downstream analysis.

Xenium Analyzer Software Versions

The table below summarizes software version requirements based on assay workflow performed.

Confirm instrument is running correct software version before initiating run. Upgrading software just prior to instrument loading is not advised.

Assay	Software version requirements		
	XA v4.0 or higher	XA v3.0	XA v2.0
Xenium In Situ Gene and Protein Expression with Cell Segmentation *(CG000819)*	✓		
Xenium Prime In Situ Gene Expression with optional Cell Segmentation *(CG000760)*	✓	✓	
Xenium In Situ Gene Expression + Cell Segmentation *(CG000749)*	✓	✓	✓
Xenium In Situ Gene Expression *(CG000582)*	✓	✓	✓

Software Overview
contd.

Xenium Explorer

The Xenium Explorer software provides off-instrument downstream analysis and visualization. Users can 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 stained microscopy images. Users can also check data quality and export or share data to inform downstream analyses.

Xenium Explorer is available for Mac or Windows computers.

Visit the 10x Genomics Support website for additional information.

Xenium Ranger

Xenium Ranger provides flexible off-instrument reanalysis of Xenium In Situ data. Relabel transcripts, resegment cells, import your own segmentation data, or rename datasets. Results can be visualized in Xenium Explorer.

Visit the 10x Genomics Support website for additional information.

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Tips and Best Practices

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) is retrieved from storage just prior to loading the instrument.

- Xenium slide should be in a Xenium cassette without a lid and well filled with 1,000 µl of PBS-T.

Xenium Cassette stored in PBS-T

Applies to both Xenium Cassette and Xenium Cassette v2. The image shows a Xenium Cassette for reference only

Vibration Isolation Table Specifications

Confirm that the Vibration Isolation Table gauges meet the following specifications

Vibration Isolation Table Gauge	Location	Specifications
Source air supply (Compressor, wall air, tank, etc.)	Directly in wall or where building facilities usually preconfigures	~80–150 psi
Table air supply	Right side of system that connects the wall source to vibration isolation table leg	~70–80 psi
Table leg pressure	Back left leg of vibration isolation table	~50–0 psi

If any values are out of range, contact support@10xgenomics.com for assistance

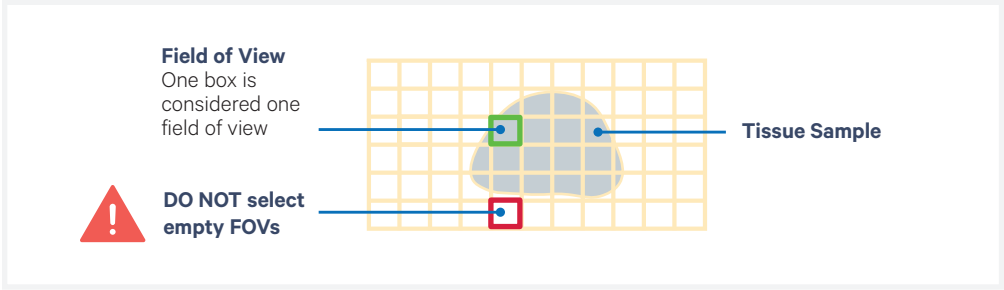
Region Selection Guidelines

During sample scanning, regions of interest must be defined prior to instrument run.

Key considerations with selecting regions

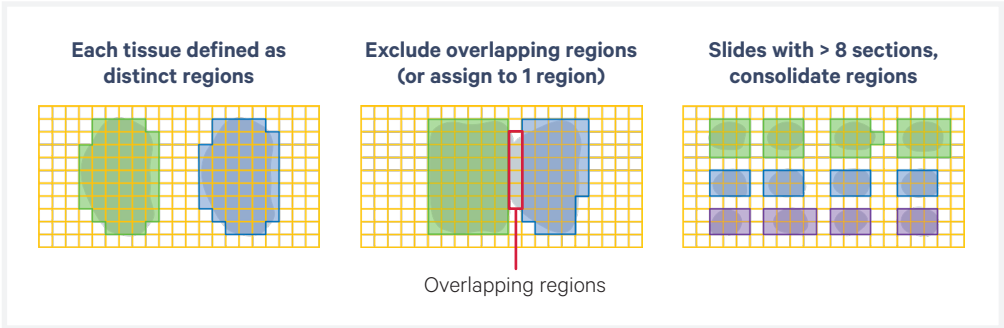
See Region Selection for full list of guidelines.

- 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.

- Avoid including FOVs that are <5% filled with tissue in a region. Inclusion of FOVs that are mostly blank can lead to stitching and registration errors
- Region names must be unique across all slides.
- **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.
  - No more than 8 regions can be selected per slide. If a slide contains more than 8 sections, consolidating some of these is recommended.



Reagent Plate and Buffer Preparation

Storing and Thawing Reagent Plates

Reagent plates are packaged in mylar bags for protection. Keep plates in mylar bag during storage and thawing. When ready to use, open the bag and remove the foil-sealed plate prior to preparation for loading.

Reagent Plates for all Xenium workflows are listed below.

Reagent Plate	Storage	Thawing Instructions
A Xenium Decoding Reagent Module A	Store at **4°C** upon receipt.	Plate is ready to use on day of instrument. No thawing or equilibration is necessary.
A Xenium Decoding Reagent Module A (Universal)		
A Xenium Prime Decoding Reagent Module A		
B Xenium Decoding Reagent Module B	Store at **-20°C** upon receipt	Thaw at **4°C** for **16–72 h prior** to handling and loading onto the instrument. For same day use, thaw plate at **37°C** water bath for **2.5 h** in mylar packaging. Factor in the thawing step when planning an experiment.
B Xenium Prime Decoding Reagent Module B - 5K		
C Xenium Cell Segmentation Detection Module		
C Xenium Protein Detection Reagents		

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 the Reagent Preparation section.

Buffers differ based on assay performed. Follow the appropriate reagent preparation protocol. Failure to prepare the correct buffers will result in failed instrument run.

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/unreliable data generation.

Following 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.

Cleaning Cassette Carrier

Raised areas indicate contact with slide.

Areas must be free of lint and liquid for successful run.

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.

Follow local lab safety or EHS requirements for using compressed air.

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. 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.

Instrument Setup
contd.

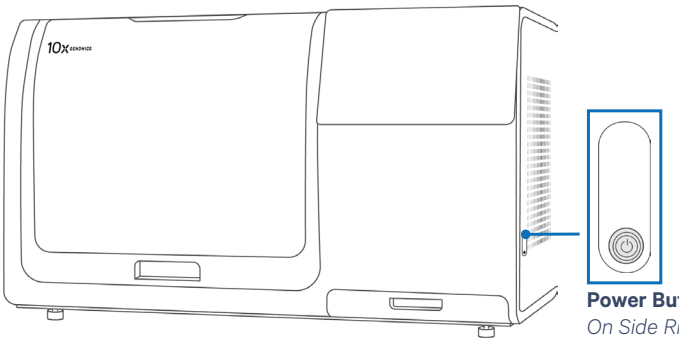
The user should not operate the switches at the back panel. The breaker switch and the main power switch on the back panel will be activated/used only during instrument installation and service.

Turn on the System

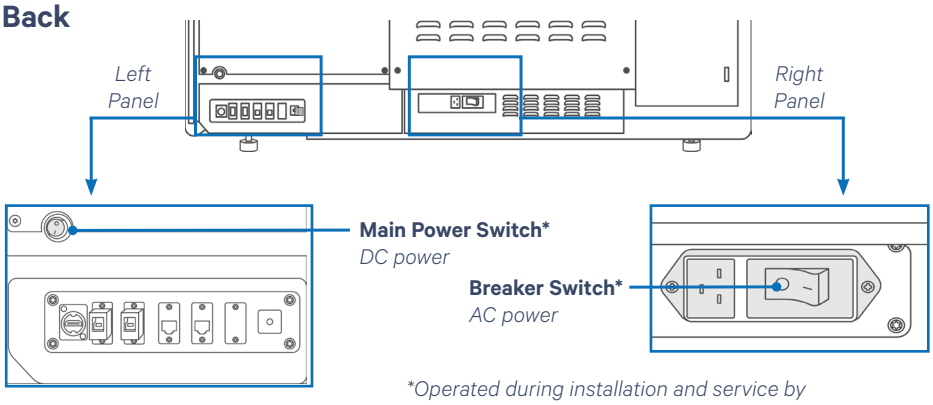
a. Power on the instrument using the power button (press **>3 sec**) on the side panel (right). See detailed information below.

- **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.

Front



Back



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

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.

Set up and Register Instrument (First-time Use Only)

a. Click Start Set Up to begin.

b. Follow on-screen instructions to set up the following

i. **Local time zone**: All times displayed on the instrument will be local time, including estimated run completion.

ii. **Network connection**: Internet connection is recommended for best support experience.

iii. **Instrument registration to 10x Genomics Cloud**: If necessary, multiple users can be registered to a single instrument.

iv. **Network shared folder (Optional)**: Used for transferring data from the instrument to local storage.

c. Upon completion, the Xenium Analyzer home screen will appear.

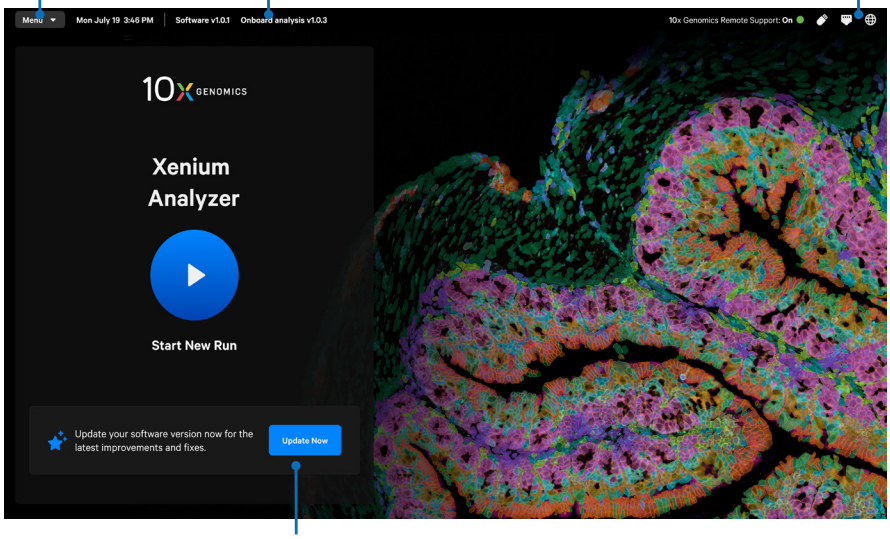
Touchscreen Menu Options

Xenium Analyzer Application Home Screen

Menu Options
Press to navigate set of options

Software Versions
Displays current instrument software version

Status Icons
Displays connected devices & network status



Software Update
Indicates new software version is available

Touchscreen Menu Options *contd.*

Status Icons

	Icon	Connection Status
USB		USB connected
	No Icon	USB not connected
Ethernet		Ethernet connected
		Ethernet connection error (Action required)
		Ethernet not connected
10x Cloud		10x Cloud connected
		10x Cloud connection error (Action required)

Settings (Menu > Open Settings)

Setting	Description
Runs	View and manage run information and export run data *(Deleted data is not visible)*
Custom Panels	View, manage, and upload custom or add-on panels here *(Only panels uploaded to the instrument are displayed)*
Network Shared Folder	Configure and manage network shared folders for output data transfer
Readiness Tests	Initiate instrument tests and view results *(The only user-run test is the Readiness Test while others on the instrument are for 10x Field Engineers only)*
Test Logs	Running log of initiated readiness tests performed
About	Information about the system (including instrument serial number), analytics, 10x Cloud registration status, storage capacity, privacy, and software versions
Software	View and update instrument software version *(Most recent release notes are accessible here)*
Network Settings	Configure network settings for instrument
Connectivity	Enable/disable remote access by 10x Genomics
Time	Configure time zone for instrument to display local time
Control Panel	View and manage instrument front panel status

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.

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

Software Updates

For version 1.6 or higher

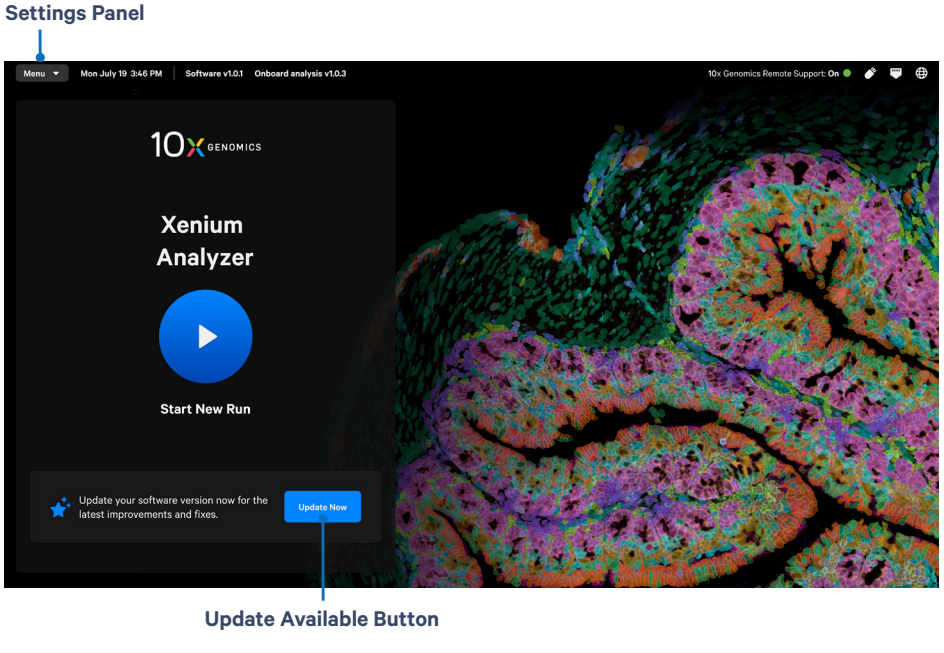
Keep the Xenium Analyzer application up to date to ensure the instrument can utilize the latest assay products, features, and bug fixes. Updates may improve or alter the on-instrument analysis pipeline.

Using the latest version of the software is recommended, however changes to the pipeline may introduce batch effects if upgrades are completed between runs involved in a multirun experiment. Consult the Xenium Software Release Notes for details before updating.

Instrument requires internet connection to receive 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.



Software Updates
contd.

From Home Screen

a. When software update is available, a blue "Update Now" button will appear. Click button and follow on-screen instructions to download and install.

If Remind Me Later is selected, the Update Now message on the home screen will reappear later. Keep your system up to date to ensure latest instrument improvements and bug fixes.

From Settings Panel

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

b. If software update is available, click View Release Notes and Download to download update.

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.

Installing Software Update

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

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

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

d. Run Readiness Test (See section below for instructions).

Readiness Test

The Readiness Test verifies all systems are working optimally and the instrument is ready for use. It is included as a prerun verification for all runs, but can be initiated as a standalone operation at the discretion of the end user from the Tests Menu option. No reagents required.

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

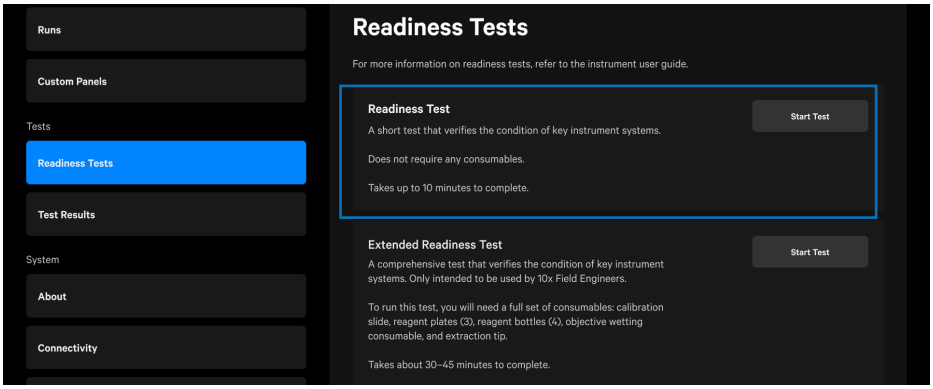
b. Under the Tests category, select Readiness Tests.

c. Three types of Readiness Tests will appear. Users run "Readiness Test". To start the test, click "Start Test" under Readiness Test.

Additional types of readiness tests available on the instrument are for 10x Field Engineers only.

Readiness Test

Select "Readiness test" to check instrument parameters



d. A successful Readiness Test verifies the instrument is ready for use. Follow on-screen instructions if a failed or incomplete test occurs.

e. Exit by selecting the Close settings button at the top-left corner of the screen, or select the Menu drop down and click Close settings.

10x GENOMICS

Reagent Preparation & Loading for Xenium v1 Gene Expression Workflows

Upcoming Changes to Xenium Decoding Reagent Module A

Xenium Decoding Reagent Module A (PN-1000624) will be replaced with Xenium Decoding Reagent Module A (Universal) (PN-1000859).

Both Xenium Decoding Reagent Module A and Xenium Decoding Reagent Module A (Universal) are compatible with Xenium In Situ Gene Expression and Xenium In Situ Gene Expression with Cell Segmentation workflows with no impact on assay performance.

If applicable, use any remaining Xenium Decoding Reagent Module A plates first.

Protocol Steps & Timing

For Xenium v1 Workflows

(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*
Thaw Cell Segmentation Detection Module**	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*
***If performing Xenium In Situ Gene Expression with Cell Segmentation Staining workflow (CG000749)*

Reagent Kits & Consumables

Kits below are used for Xenium v1 workflows only.

Instrument runs with slides prepared for Xenium In Situ Gene Expression with Cell Segmentation require all three plates.

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

**Use during for sample preparation prior to loading the instrument (CG000578, CG000579).*

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

Items	#	Part Number
A Xenium Decoding Reagent Module A OR Xenium Decoding Reagent Module A (Universal) (store at 4°C)	1	1000624 OR 1000859
B Xenium Decoding Reagent Module B (store at -20°C)	1	1000625

Consult the SDS for instructions on proper handling and disposal of volatile and hazardous chemicals.

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

Visually inspect the mylar packaging of Xenium Decoding Module A OR Xenium Decoding Module A (Universal) upon receipt to ensure it is vacuum sealed. If it is compromised, use another package and contact support@10xgenomics.com.

Xenium Cell Segmentation Detection Reagents (1 run, 2 slides) PN-1000639

Items	#	Part Number
C Xenium Cell Segmentation Detection Module (store at -20°C)**	1	1000639

***For Xenium In Situ Gene Expression with Cell Segmentation Staining (CG000749) workflow*

Reagent Plate Preparation

The following section describes reagent plate preparation for **Xenium v1 workflows** only.

TIPS

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

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

Item	10x PN	Preparation & Handling	Storage
Maintain on ice/4°C			
☐ A Xenium Decoding Reagent Module A OR Xenium Decoding Reagent Module A (Universal)	1000624 OR 1000859	-	4°C
Maintain at room temperature			
☐ B 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
☐ C Xenium Cell Segmentation Detection Module* **If performing Xenium In Situ Gene Expression Cell Segmentation Workflow*	1000639	Thaw in sealed mylar bag at 4°C for 16–72 h or at 37°C for 2.5 h	-20°C
Obtain			
☐ Deep-well, 96 well plate for counterbalancing	-	-	Ambient
☐ Centrifuge compatible with deep-well 96 well plates (~2 ml vol.) *(Allegra® X-14 Series Benchtop centrifuge 120 V or equivalent)*	-	-	Ambient
☐ Serological Pipettes	-	-	Ambient
☐ Plate seal	-	-	Ambient
☐ Laboratory Balance	-	-	Ambient

This list may not include some standard laboratory equipment.

Reagent Plate Preparation *contd.*

A Xenium Decoding Reagent Module A or Xenium Decoding Reagent Module A (Universal)

*Module A is oxygen sensitive! Keep plate in its original vacuum sealed mylar packaging during storage at **4°C**.*

a. Day of run: Open the mylar packaging and remove plate. Do not remove the foil seal 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 counterbalancing plate. See instructions on the next page.

c. Place the reagent plate and counterbalancing plate in a swinging bucket centrifuge. Once balanced, centrifuge at **1600 rcf** for **10 min** at **room temperature**.

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

B C Xenium Decoding Reagent Module B / Xenium Cell Segmentation Detection Module*

**If performing Xenium In Situ Gene Expression with Cell Segmentation Staining workflow*

*Keep plates in its original vacuum sealed mylar packaging during storage at **-20°C** and during thaw at **4°C**.*

a. Thaw plate in its original packaging at **4°C** for **16–72 h** or at **37°C** for **2.5 h**. Unopened plate in its original mylar packaging may be kept at **4°C** for up to **3 days**.

b. Equilibrate thawed plate at **room temperature** for **30 min**.

c. Open the mylar packaging to remove plate. Do not remove foil seal at any time. Mix by gently inverting the plate 20X without introducing bubbles. DO NOT vortex. Maintain at **room temperature**.

d. Prepare counterbalancing plate. See instructions on the next page.

e. Place reagent and counterbalancing plates in a swinging bucket centrifuge. 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.

Reagent Plate Preparation *contd.*

Reagent Plate Preparation Summary for Xenium v1 Workflows		
Step	**A Xenium Decoding Reagent Module A OR Xenium Decoding Reagent Module A (Universal)**	**B Xenium Decoding Reagent Module B** **C Xenium Cell Segmentation Detection Module***
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	**1600 rcf** for **10 min** at **room temperature**	**300 rcf** for **1 min** at **room temperature**
Before loading	Maintain at **4°C**	Maintain at **room temperature**

**If performing Xenium In Situ Gene Expression with Cell Segmentation Staining workflow*

Plate Counterbalancing Instructions

Reagent and Detection Modules do not weigh the same and should be counterbalanced separately.

- Weigh the Xenium module plate with lid on *(example: 190 g)*.
- Place the empty counterbalancing deep-well 96 well plate on the weighing balance. Pipette (multichannel/serological) 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.

Buffer Preparation

The following section describes buffer preparation for **Xenium v1 workflows** only.

Item	10x PN	Composition	Storage
Obtain and Fill			
☐ 1 Deionized Water/Xenium Instrument Wash Buffer	3001198	Milli-Q Water	Ambient
☐ 2 Xenium Sample Wash Buffer A	3001199	PBS + Tween	Ambient
☐ 3 Xenium Sample Wash Buffer B	3001200	Milli-Q Water	Ambient
☐ 4 Xenium Probe Removal Buffer	3001201	DMSO + Tween + KCl	Ambient
Obtain			
☐ Nuclease-free Water (not DEPC-treated) or Nuclease-free Milli-Q water (Biopak® Polisher)	-	-	Ambient
☐ PBS-Tween **OR** PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free and Tween 20 Detergent Solution (10% solution)			Ambient
☐ Serological Pipettes (10 ml, 25 ml, 50 ml) & Serological Pipette Controller	-	-	Ambient
☐ Glass Bottles with Cap (500 ml, 1 L)	-	-	Ambient
☐ Potassium Chloride (KCl)	-	-	Ambient
☐ 100% DMSO	-	-	Ambient
☐ Pipette Tips (1,000 µl) & Pipette	-	-	Ambient

Choose only one for Xenium Sample Wash Buffer A

This list may not include some standard laboratory equipment.

Buffer Preparation *contd.*

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

Before 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 cylinder 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** Nuclease-free Water/Nuclease-free Milli-Q Water and cap bottle with standard bottle cap.

2 Xenium Sample Wash Buffer A

Prepare 1X PBS-T according to the table below in a glass bottle and maintain at **room temperature**. Add reagents in the order listed. Cap bottle and invert gently to mix. Ensure minimal bubbles are introduced.

If preparing from powder:

Reagents *Add reagents in order listed*	PN	Xenium v1
Nuclease-free Water	AM9932 or CDUFBI0A1	1 L
PBS-Tween *(choose one)*	P3563-10PAK	1 Pack
	PPB005-20PAK	2 Packs
Total	—	**1 L**

If preparing from liquid:

Reagents *Add reagents in order listed*	PN	Stock	Final	Xenium v1
Nuclease-free Water	AM9932 or CDUFBI0A1	-	-	895 ml
PBS	AM9624	10X	1X	100 ml
Tween 20	28320	10%	0.05%	5 ml
Total	—			**1 L**

Buffer Preparation
contd.

3 Xenium Sample Wash Buffer B

Fill Reagent Bottle #3 with **150 ml** Nuclease-free Water/Nuclease-free Milli-Q Water and cap bottle with standard bottle cap.

4 Xenium Probe Removal Buffer

Prepare Probe Removal Buffer according to the table below in a glass bottle. Add reagents in the order listed. Cap bottle and invert gently to mix. Ensure minimal bubbles are introduced. Maintain at **room temperature** for **30 min** to cool it down and to clear bubbles created during mixing. Minor amounts of bubbles are acceptable.

Probe Removal Buffer for Xenium v1 Workflow *Add reagents in order listed*	**Stock**	**Final**	**1X (ml)**
Nuclease-free Water	—	—	139.5
DMSO	100%	50%	150
KCl	2,000 mM	50 mM	7.5
Tween 20	10%	0.1%	3
Total	—	—	**300**

Buffer may become warm during preparation.

DMSO is hazardous and handled inside a fume hood. Consult the SDS for instructions on proper handling and disposal.

Reagent Plate Loading

Number of plates depends on assay performed. Touchscreen instructions will reflect correct number based on assay selected. Ensure all required plates are loaded.

a. Remove lid from reagent plates.

b. Place the reagent plates into their respective positions. (See image below) Firmly press plates down, and rock gently back and forth as needed, until plates are completely level.

Xenium v1 Reagent Plate Loading

Plate Orientation

Load plate with A1 well at the top left corner as shown. Confirm plates are placed in the correct location. Improper placement will result in a failed run.

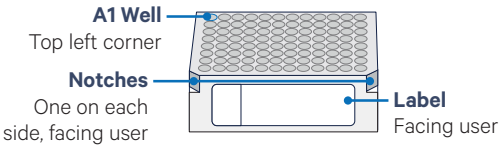
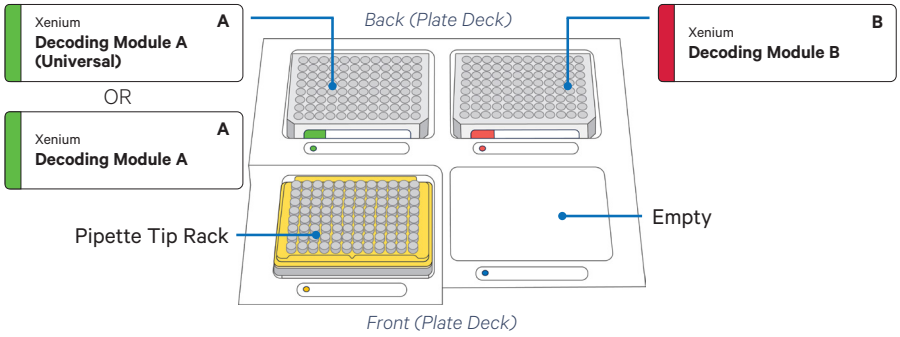
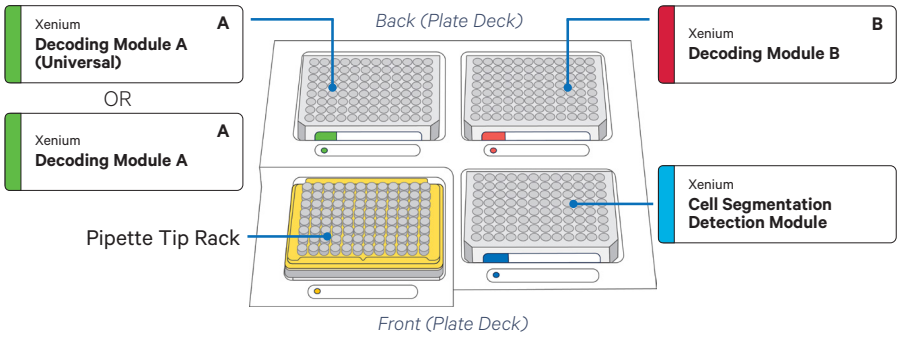


Plate Loading

Xenium In Situ Gene Expression *(CG000582)*



Xenium In Situ Gene Expression with Cell Segmentation Staining *(CG000749)*



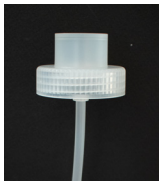
Reagent Bottle Loading

a. Replace standard bottle cap with a Xenium Buffer Cap (included in the Xenium Decoding Consumables kit).

Xenium Buffer Cap

(included in the Xenium Decoding Consumables kit)

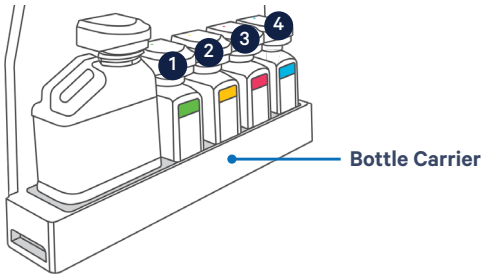
For each reagent bottle, replace the standard reagent bottle cap with a Xenium Buffer Cap prior to loading onto the instrument



b. Place bottles in the bottle carrier in the designated order.

Match bottle position color and number with label on reagent bottle for accurate placement. Incorrect placement will result in a failed instrument run.

Reagent Bottle Positions in the Bottle Carrier



Reagent Bottle Buffer	Xenium v1
1 Deionized Water/Xenium Instrument Wash Buffer	500 ml Nuclease-free water
2 Xenium Sample Wash Buffer A	1 L PBS-T
3 Xenium Sample Wash Buffer B	150 ml Nuclease-free water
4 Xenium Probe Removal Buffer	300 ml 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. Place empty uncapped Waste Bottle 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.

f. Proceed to System Operation.

10x GENOMICS

Reagent Preparation & Loading for Xenium Prime Gene Expression Workflow

Upcoming Changes to Xenium Decoding Reagent Module A

Xenium Prime Decoding Reagent Module A (PN-1000721) will be replaced with Xenium Decoding Reagent Module A (Universal) (PN-1000859).

Both Xenium Prime Decoding Reagent Module A and Xenium Decoding Reagent Module A (Universal) are compatible with the Xenium Prime In Situ Gene Expression workflow with no impact on assay performance.

If applicable, use any remaining Xenium Prime Decoding Reagent Module A plates first.

Protocol Steps & Timing

For Xenium Prime Workflows

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

Steps	Timing	
	Hands-on Time	Total Time
Day 1		
Thaw Xenium Prime Decoding Reagent Module B - 5K	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	-	2-6 days
Post-Run Cleanup	5 min	10 min

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

Reagent Kits & Consumables

Kits below are used for the **Xenium Prime workflow** only.

No additional decoding reagent plates are required if performing cell segmentation staining.

Xenium Decoding Consumables v2 (1 run, 2 slides) PN-1000726

Items *(store at room temperature)*	#	Part Number
Xenium Cassette Kit v2* (2 rxns) • includes 2 Cassettes v2, 8 Cassette Lids v2, and 4 Cassette Inserts	1	1000723
Extraction Tip	1	2000757
Pipette Tips	1	3000866
Xenium Buffer Cap	4	3000949
Xenium Objective Wetting Consumable	1	2000749

**Required for sample preparation, which is performed prior to loading the instrument (Documents CG000578, CG000579).*

Xenium Reagent Bottles PN-1000730

Items *(store at room temperature)*	#	Part Number
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

Xenium Prime 5K Decoding Reagents (2 rxns) PN-1000740

Consult the SDS for instructions on proper handling and disposal of volatile and hazardous chemicals.

Items	#	Part Number
A Xenium Prime Decoding Reagent Module A OR Xenium Decoding Reagent Module A (Universal) (store at 4°C)	1	1000721 OR 1000859
B Xenium Prime Decoding Reagent Module B - 5K (store at -20°C)	1	1000722

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.

Visually inspect the mylar packaging of Xenium Prime Decoding Module A OR Xenium Decoding Module A (Universal) upon receipt to ensure it is vacuum sealed. If it is compromised, use another package and contact support@10xgenomics.com

Reagent Plate Preparation

The following section describes reagent plate preparation for the **Xenium Prime workflow** only.

TIPS

*Xenium Prime 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.*

Consult the SDS for instructions on proper handling and disposal of volatile and hazardous chemicals.

Item	10x PN	Preparation & Handling	Storage
Maintain on ice/4°C			
☐ A Xenium Prime Decoding Reagent Module A OR Xenium Decoding Reagent Module A (Universal)	1000721 OR 1000859	-	4°C
Maintain at room temperature			
☐ B Xenium Prime Decoding Reagent Module B - 5K	1000722	Thaw in sealed mylar bag at 4°C for 16–72 h or at 37°C for 2.5 h	-20°C
Obtain			
☐ Deep-well, 96 well plate for counterbalancing	-	-	Ambient
☐ Centrifuge compatible with deep-well 96 well plates (~2 ml vol.) *(Allegra® X-14 Series Benchtop centrifuge 120 V or equivalent)*	-	-	Ambient
☐ Serological Pipettes	-	-	Ambient
☐ Plate seal	-	-	Ambient
☐ Laboratory Balance	-	-	Ambient

This list may not include some standard laboratory equipment.

Reagent Plate Preparation *contd.*

A Xenium Prime Decoding Reagent Module A or Xenium Decoding Reagent Module A (Universal)

*Module A is oxygen sensitive! Keep plate in its original vacuum sealed mylar packaging during storage at **4°C**.*

a. Day of run: Open the mylar packaging and remove plate. Do not remove foil seal at any time. Mix by gently inverting the plate 20X without introducing bubbles. DO NOT vortex. Maintain on ice.

Plate must be used within 7.5 days (includes run time) after opening and removal from mylar packaging.

b. Prepare counterbalancing plate. See instructions on the next page.

c. Place the reagent plate and counterbalancing plate in a swinging bucket centrifuge. Once balanced, centrifuge at **1600 rcf** for **10 min** at **room temperature**.

d. Remove from centrifuge and place reagent plate at **4°C or on ice** until loading. DO NOT invert the plate after centrifugation.

B Xenium Prime Decoding Reagent Module B

*Keep plates in its original vacuum sealed mylar packaging during storage at **-20°C** and during thaw at **4°C**.*

a. Thaw plate in its original packaging at **4°C** for **16–72 h** or at **37°C** for **2.5 h**. Unopened plate in its original mylar packaging may be kept at **4°C** for up to **3 days**.

b. Equilibrate thawed plate at **room temperature** for **30 min**.

c. Open the mylar packaging to remove plate. Do not remove foil seal at any time. Mix by gently inverting the plate 20X without introducing bubbles. DO NOT vortex. Maintain at **room temperature**.

d. Prepare counterbalancing plate. See instructions on the next page.

e. Place reagent and counterbalancing plates in a swinging bucket centrifuge. 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.

Reagent Plate Preparation *contd.*

Reagent Plate Preparation Summary for Xenium Prime Workflow		
Step	**A Xenium Prime Decoding Reagent Module A OR Xenium Decoding Reagent Module A (Universal)**	**B Xenium Prime Decoding Reagent Module B - 5K**
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	**1600 rcf** for **10 min** at **room temperature**	**300 rcf** for **1 min** at **room temperature**
Before loading	Maintain at **4°C**	Maintain at **room temperature**

Plate Counterbalancing Instructions

Reagent and Detection Modules do not weigh the same and should be counterbalanced separately.

- Weigh the Xenium module plate with lid on. *(example: 190 g)*.
- Place the empty counterbalancing deep-well 96 well plate on the weighing balance. Pipette (multichannel/serological) 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.

Buffer Preparation

The following section describes buffer preparation for the **Xenium Prime workflow** only.

Item	10x PN	Composition	Storage
Obtain and Fill			
☐ 1 Deionized Water/Xenium Instrument Wash Buffer	3001198	Milli-Q Water	Ambient
☐ 2 Xenium Sample Wash Buffer A	3001199	PBS + Tween	Ambient
☐ 3 Xenium Sample Wash Buffer B	3001200	PBS + Tween	Ambient
☐ 4 Xenium Probe Removal Buffer	3001201	DMSO + Tween + KCl	Ambient
Obtain			
☐ Nuclease-free Water (not DEPC-treated) or Nuclease-free Milli-Q water (Biopak® Polisher)	-	-	Ambient
☐ PBS-Tween **OR** PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free and Tween 20 Detergent Solution (10% solution)			Ambient
☐ Serological Pipettes (10 ml, 25 ml, 50 ml) & Serological Pipette Controller	-	-	Ambient
☐ Glass Bottles with Cap (500 ml, 1 L)	-	-	Ambient
☐ Potassium Chloride (KCl)	-	-	Ambient
☐ 100% DMSO	-	-	Ambient
☐ Pipette Tips (1,000 µl) & Pipette	-	-	Ambient

Choose only one for Xenium Sample Wash Buffer A/B

This list may not include some standard laboratory equipment.

Buffer Preparation *contd.*

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

Before 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 cylinder 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** Nuclease-free Water/Nuclease-free Milli-Q Water and cap bottle with standard bottle cap.

2 3 Xenium Sample Wash Buffer A and B

Prepare 1X PBS-T according to the table below in a glass bottle and maintain at **room temperature**. Add reagents in the order listed. Cap bottle and invert gently to mix. Ensure minimal bubbles are introduced.

If preparing from powder:

Reagents *Add reagents in order listed*	PN	Xenium Prime
Nuclease-free Water	AM9932 or CDUFBI0A1	2 L
PBS-Tween *(choose one)*	P3563-10PAK	2 Pack
	PPB005-20PAK	4 Packs
Total	—	**2 L**

If preparing from liquid:

Reagents *Add reagents in order listed*	PN	Stock	Final	Xenium Prime
Nuclease-free Water	AM9932 or CDUFBI0A1	-	-	1,790 ml
PBS	AM9624	10X	1X	200 ml
Tween 20	28320	10%	0.05%	10 ml
Total	—			**2 L**

Fill Reagent Bottle #2 and #3 with **1 L** PBS-T each and cap with standard bottle cap.

Buffer Preparation *contd.*

4 Xenium Probe Removal Buffer

Prepare Probe Removal Buffer according to the table below in a glass bottle. Add reagents in the order listed. Cap bottle and invert gently to mix. Ensure minimal bubbles are introduced. Maintain at **room temperature** for **30 min** to cool it down and to clear bubbles created during mixing. Minor amounts of bubbles are acceptable.

Probe Removal Buffer for Xenium Prime Workflow *Add reagents in order listed*	Stock	Final	1X (ml)
Nuclease-free Water	—	—	232.5
DMSO	100%	50%	250
KCl	2,000 mM	50 mM	12.5
Tween 20	10%	0.1%	5
Total	—	—	**500**

DMSO is hazardous and handled inside a fume hood. Consult the SDS for instructions on proper handling and disposal.

Buffer may become warm during preparation.

Reagent Plate Loading

a. Remove lid from reagent plates.

b. Place the reagent plates into their respective positions. (See image below) Firmly press plates down, and rock gently back and forth as needed, until plates are completely level.

Xenium Prime Reagent Plate Loading

Plate Orientation

Load plate with A1 well at the top left corner as shown. Confirm plates are placed in the correct location. Improper placement will result in a failed run.

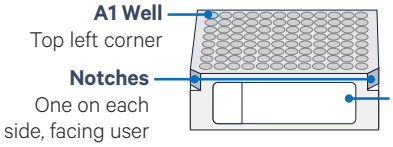
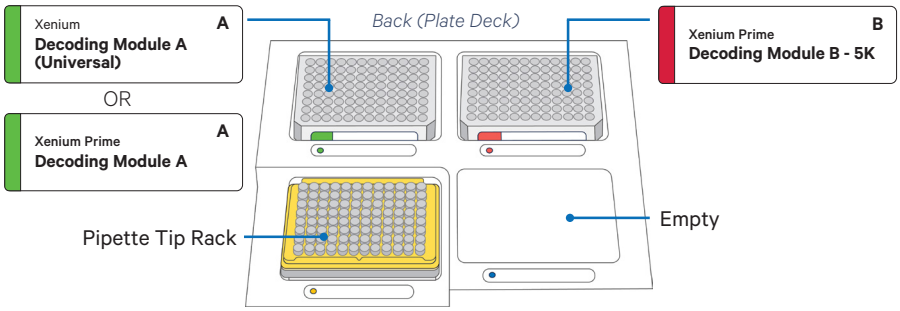


Plate Loading



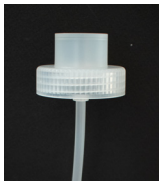
Reagent Bottle Loading

a. Replace standard bottle cap with a Xenium Buffer Cap (included in the Xenium Decoding Consumables kit).

Xenium Buffer Cap

(included in the Xenium Decoding Consumables kit)

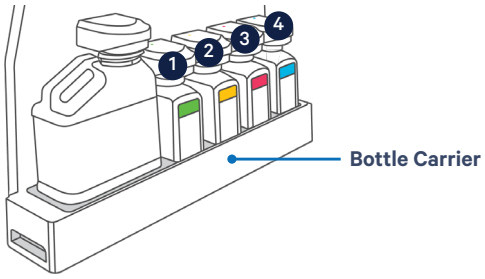
For each reagent bottle, replace the standard reagent bottle cap with a Xenium Buffer Cap prior to loading onto the instrument



b. Place bottles in the bottle carrier in the designated order.

Match bottle position color and number with label on reagent bottle for accurate placement. Incorrect placement will result in a failed instrument run.

Reagent Bottle Positions in the Bottle Carrier



Reagent Bottle Buffer	Xenium Prime
1 Deionized Water/Xenium Instrument Wash Buffer	500 ml Nuclease-free water
2 Xenium Sample Wash Buffer A	1 L PBS-T
3 Xenium Sample Wash Buffer B	1 L PBS-T
4 Xenium Probe Removal Buffer	500 ml 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. Place empty uncapped Waste Bottle 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.

f. Proceed to System Operation.

10x GENOMICS

Reagent Preparation & Loading for Xenium Gene & Protein Expression Workflow

Protocol Steps & Timing

For Xenium Gene & Protein Workflow

(on-instrument; for FFPE samples)

Steps	Timing	
	Hands-on Time	Total Time
Day 1		
Thaw Decoding Reagent Module B	5 min	16–72 h at 4°C*
Thaw Protein Detection Reagents	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	-	2-6 days
Post-Run Cleanup	5 min	10 min

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

Reagent Kits & Consumables

Kits below are used for the **Xenium Protein workflow** only.

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

**Use during for sample preparation prior to loading the instrument (CG000578, CG000579).*

Xenium RNA & Protein Detection Reagents (1 run, 2 slides) PN-1000884

Consult the SDS for instructions on proper handling and disposal of volatile and hazardous chemicals.

Items	#	Part Number
A Xenium Decoding Reagent Module A (Universal) (store at 4°C)	1	1000859
B Xenium Decoding Reagent Module B (store at -20°C)	1	1000625
C Xenium Protein Detection Reagents (store at -20°C)	1	1000852

Storage conditions differ by reagent plate. Refer to packaging for proper storage instructions upon receipt. Failure to comply with storage instructions will render reagents unusable.

Visually inspect the mylar packaging of Decoding Module A (Universal) upon receipt to ensure it is vacuum sealed. If it is compromised, use another package and contact support@10xgenomics.com.

Reagent Plate Preparation

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

The following section describes reagent plate preparation for the **Xenium Protein workflow** only.

TIPS

*Xenium Decoding Reagent Module B and Xenium Protein Detection Reagents require overnight thawing at **4°C**. Ensure plate is removed from **-20°C** and placed at **4°C** the night prior to the instrument run.*

Xenium Protein workflow is only compatible with Xenium Decoding Reagent Module A (Universal). Confirm the correct plate is prepared and loaded.

Do NOT load other Xenium Decoding Reagent Module A plates for a Xenium Protein run.

Item	10x PN	Preparation & Handling	Storage
Maintain on ice/4°C			
☐ A Xenium Decoding Reagent Module A (Universal)	1000859	-	4°C
Maintain at room temperature			
☐ B 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
☐ C Xenium Protein Detection Reagents	1000852	Thaw in sealed mylar bag at 4°C for 16–72 h or at 37°C for 2.5 h	-20°C
Obtain			
☐ Deep-well, 96 well plate for counterbalancing	-	-	Ambient
☐ Centrifuge compatible with deep-well 96 well plates (~2 ml vol.) *(Allegra® X-14 Series Benchtop centrifuge 120 V or equivalent)*	-	-	Ambient
☐ Serological Pipettes	-	-	Ambient
☐ Plate seal	-	-	Ambient
☐ Laboratory Balance	-	-	Ambient

This list may not include some standard laboratory equipment.

Reagent Plate Preparation *contd.*

A Xenium Decoding Reagent Module A (Universal)

*Module A is oxygen sensitive! Keep plate in its original vacuum sealed mylar packaging during storage at **4°C**.*

a. **Day of run**: Open the mylar packaging and remove plate. Do not remove the foil seal 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 counterbalancing plate. See instructions on the next page.

c. Place the reagent plate and counterbalancing plate in a swinging bucket centrifuge. Once balanced, centrifuge at **1600 rcf** for **10 min** at **room temperature**.

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

B C Xenium Decoding Reagent Module B / Xenium Protein Detection Reagents

*Keep plates in its original vacuum sealed mylar packaging during storage at **-20°C** and during thaw at **4°C**.*

a. Thaw plate in its original packaging at **4°C** for **16–72 h** or at **37°C** for **2.5 h**. Unopened plate in its original mylar packaging may be kept at **4°C** for up to **3 days**.

b. Equilibrate thawed plate at **room temperature** for **30 min**.

c. Open the mylar packaging to remove plate. Do not remove foil seal at any time. Mix by gently inverting the plate 20X without introducing bubbles. DO NOT vortex. Maintain at **room temperature**.

d. Prepare counterbalancing plate. See instructions on the next page.

e. Place reagent and counterbalancing plates in a swinging bucket centrifuge. 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.

Reagent Plate Preparation *contd.*

Reagent Plate Preparation Summary for Xenium Protein Workflows		
Step	**A Xenium Decoding Reagent Module A (Universal)**	**B Xenium Decoding Reagent Module B** **C Xenium Protein Detection Reagents**
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	**1600 rcf** for **10 min** at **room temperature**	**300 rcf** for **1 min** at **room temp.**
Before loading	Maintain at **4°C**	Maintain at **room temperature**

Plate Counterbalancing Instructions

Reagent and Detection Modules 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.

Buffer Preparation

The following section describes buffer preparation for the **Xenium Protein workflow** only.

Item	10x PN	Composition	Storage
Obtain and Fill			
☐ 1 Deionized Water/Xenium Instrument Wash Buffer	3001198	Milli-Q Water	Ambient
☐ 2 Xenium Sample Wash Buffer A	3001199	PBS + Tween	Ambient
☐ 3 Xenium Sample Wash Buffer B	3001200	PBS + Tween	Ambient
☐ 4 Xenium Probe Removal Buffer	3001201	DMSO + Tween + KCl	Ambient
Obtain			
☐ Nuclease-free Water (not DEPC-treated) or Nuclease-free Milli-Q water (Biopak® Polisher)	-	-	Ambient
☐ PBS-Tween **OR** PBS - Phosphate Buffered Saline (10X) pH 7.4, RNase-free and Tween 20 Detergent Solution (10% solution)			Ambient
☐ Serological Pipettes (10 ml, 25 ml, 50 ml) & Serological Pipette Controller	-	-	Ambient
☐ Glass Bottles with Cap (500 ml, 1 L)	-	-	Ambient
☐ Potassium Chloride (KCl)	-	-	Ambient
☐ 100% DMSO	-	-	Ambient
☐ Pipette Tips (1,000 µl) & Pipette	-	-	Ambient

Choose only one for Xenium Sample Wash Buffer A

This list may not include some standard laboratory equipment.

Buffer Preparation *contd.*

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

Before 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 cylinder 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 Nuclease-free Water/Nuclease-free Milli-Q Water and cap bottle with standard bottle cap.

2 3 Xenium Sample Wash Buffer A and B

Prepare 1X PBS-T according to the table below in a glass bottle and maintain at **room temperature**. Add reagents in the order listed. Cap bottle and invert gently to mix. Ensure minimal bubbles are introduced.

If preparing from powder:

Reagents *Add reagents in order listed*	PN	Xenium Protein
Nuclease-free Water	AM9932 or CDUFBI0A1	2 L
PBS-Tween *(choose one)*	P3563-10PAK	2 Pack
	PPB005-20PAK	4 Packs
Total	—	**2 L**

If preparing from liquid:

Reagents *Add reagents in order listed*	PN	Stock	Final	Xenium Protein
Nuclease-free Water	AM9932 or CDUFBI0A1	-	-	1790 ml
PBS	AM9624	10X	1X	200 ml
Tween 20	28320	10%	0.05%	10 ml
Total	—			**2 L**

Fill Reagent Bottle #2 and #3 with **1 L** PBS-T each and cap with standard bottle cap.

Buffer Preparation
contd.

4 Xenium Probe Removal Buffer

Prepare Probe Removal Buffer according to the table below in a glass bottle. Add reagents in the order listed. Cap bottle and invert gently to mix. Ensure minimal bubbles are introduced. Maintain at **room temperature** for **30 min** to cool it down and to clear bubbles created during mixing. Minor amount of bubbles are acceptable.

Probe Removal Buffer for Xenium Protein Workflow *Add reagents in order listed*	**Stock**	**Final**	**1X (ml)**
Nuclease-free Water	—	—	232.5
DMSO	100%	50%	250
KCl	2,000 mM	50 mM	12.5
Tween 20	10%	0.1%	5
Total	—	—	**500**

Buffer may become warm during preparation.

DMSO is hazardous and handled inside a fume hood. Consult the SDS for instructions on proper handling and disposal.

Reagent Plate Loading

Number of plates depends on assay performed. Touchscreen instructions will reflect correct number based on assay selected. Ensure all required plates are loaded.

a. Remove lid from reagent plates.

b. Place the reagent plates into their respective positions (see image below). Firmly press plates down, and rock gently back and forth as needed, until plates are completely level.

Xenium Protein Reagent Plate Loading

Plate Orientation

Load plate with A1 well at the top left corner as shown. Confirm plates are placed in the correct location. Improper placement will result in a failed run.

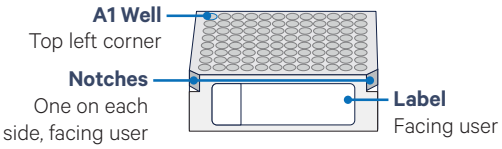
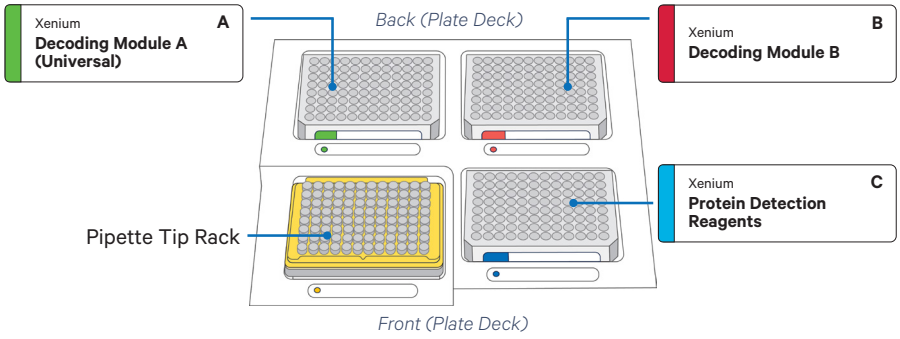


Plate Loading

Xenium Protein workflow is only compatible with Xenium Decoding Reagent Module A (Universal). Do NOT load other Xenium Decoding Reagent Module A plates for a Xenium Protein run.



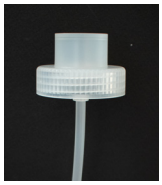
Reagent Bottle Loading

a. Replace standard bottle cap with a Xenium Buffer Cap (included in the Xenium Decoding Consumables kit).

Xenium Buffer Cap

(included in the Xenium Decoding Consumables kit)

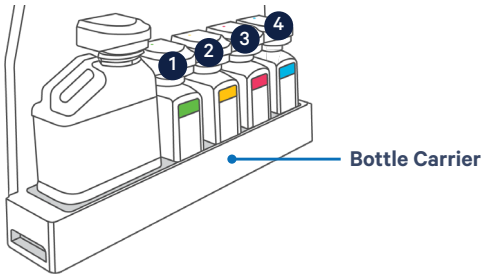
For each reagent bottle, replace the standard reagent bottle cap with a Xenium Buffer Cap prior to loading onto the instrument



b. Place bottles in the bottle carrier in the designated order.

Match bottle position color and number with label on reagent bottle for accurate placement. Incorrect placement will result in a failed instrument run.

Reagent Bottle Positions in the Bottle Carrier



Reagent Bottle Buffer	Xenium v1
1 Deionized Water/Xenium Instrument Wash Buffer	500 ml Nuclease-free water
2 Xenium Sample Wash Buffer A	1 L PBS-T
3 Xenium Sample Wash Buffer B	1 L PBS-T
4 Xenium Probe Removal Buffer	500 ml 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. Plate empty uncapped Waste Bottle 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.

f. Proceed to System Operation.

10x GENOMICS

System Operation

Initialize Instrument

Slides from different Xenium workflows cannot be run together on the same instrument run.

Detailed instructions below are for Xenium Onboard Analysis Software version 4.0 or higher. Screens may differ in previous versions. Follow on-screen instructions.

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

b. *If not already open,* launch the Xenium Analyzer Application by clicking the blue icon the touchscreen.

c. Click "Start New Run" button. System checks take **~ 3 min**.

d. Input Run Name. Run name can contain maximum 33 characters and cannot contain !@#$%&*)+=

Ensure correct selection is made. All downstream instrument functions will be affected.

e. Select which 10x assay was used to prepare samples.

Xenium v1 Gene Expression *includes the Xenium In Situ Gene Expression and Xenium In Situ Gene Expression with optional Cell Segmentation Staining workflows. Proceed to step g.*

Xenium Prime 5K Gene Expression *includes the Xenium Prime In Situ Gene Expression with optional Cell Segmentation Staining workflow. Proceed to step g.*

Xenium Gene & Protein Expression *includes the Xenium Protein In Situ Gene Expression workflow. Proceed to step f.*

f. For Xenium Gene and Protein only

TIPS *Subpanel selections are applied to both slides. Running different subpanel selections in the same instrument run is not supported.*

i. **Subpanels:** Select the protein subpanels used during assay workflow. At least one must be selected.

ii. **Individual Imaging:** Select option to enable individual imaging of cell segmentation markers. This will add additional run time.

iii. Proceed to step h.

g. For Xenium v1 and Xenium Prime -

i. **Indicate Xenium Prime**

- Select Yes if samples were prepared using Xenium Prime In Situ Gene Expression with optional Cell Segmentation Staining User Guide (CG000760).
- Select No if samples prepared using Xenium In Situ Gene Expression User Guide (CG000582) or Xenium In Situ Gene Expression with Cell Segmentation Staining User Guide (CG000749).

Initialize Instrument *contd.*

ii. **Indicate Multimodal Cell Segmentation**

- Select Yes if samples were prepared using Xenium Prime In Situ Gene Expression with optional Cell Segmentation Staining User Guide (CG000760) and performing cell segmentation staining or Xenium In Situ Gene Expression with Cell Segmentation Staining User Guide (CG000749).
- Select No if samples prepared using Xenium In Situ Gene Expression User Guide (CG000582)

Failure to select multimodal segmentation will default to nuclear expansion and multimodal data will not be recoverable.

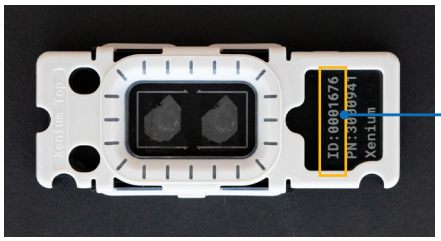
Selecting multimodal segmentation without loading Cell Segmentation Detection Module will lead to run failure and sample will not be recoverable.

h. Select Onboard Analysis Version. Running the latest version is recommended. Previous version(s) currently supported are available to select if comparison to past runs is desired.
(Xenium Gene and Protein requires Version 4.0.)

i. Add Cassette Details. If using only one slide, either of the two cassette carriers may be used.

i. **Cassette Name** (used to reference data from this cassette) Cannot contain !@#$%&*)+=

ii. **Xenium Slide ID** (7-character ID found on the bottom of the slide)



Xenium Cassette shown

Initialize Instrument *contd.*

j. Select Gene Panel

Only gene panels compatible with the workflow chosen are available to select. Click Back to revise if necessary.

Slides from different Xenium workflows cannot be run together on the same instrument run.

i. Click "Select of Upload a Panel"

ii. **To select a preloaded panel**, expand the drop-down menu to select a Pre-designed or preloaded Custom panel. Confirm desired panel is selected and click "Continue". Panel information under Panel Selection will populate.

iii. **To upload a new custom panel from USB drive**, insert USB into USB port on the Xenium Analysis Computer. Click "Upload" on instrument touchscreen and select panel file. Confirm desired panel is selected and click "Continue". Panel information under Panel Selection will populate. Once panel is uploaded, it is safe to remove the USB drive.

If using a custom panel, the Design ID on the custom panel tube label 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.*

k. If running two cassettes, load panel for remaining cassette. When all the information is populated, click "Continue".

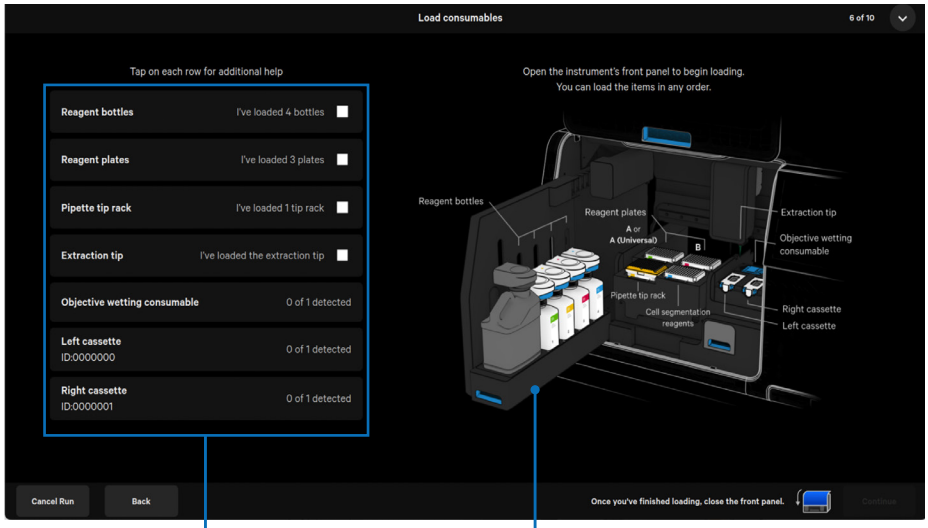
Load Consumables

Initialize ~10–20 min > **Load ~5 min** > Sample Scan 1 h > Region Sel. ~10 min > Run 2-6 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 for loading.

Actual screen may vary based on workflow selected.



Consumables
Loading status will appear here. Click for detailed instructions

Deck Directory
Displays location of consumables to aid in loading

Gather all items listed below for loading.

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

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

Load Consumables *contd.*

Reagent Bottles

See assay-specific Reagent Preparation & Loading chapter for instructions on loading reagent bottles. Reagent buffers differ by assay. Confirm appropriate buffers and buffer volumes are used.

Reagent Bottle Buffer	Xenium v1	Xenium Prime or Xenium Protein
1 Deionized Water/Xenium Instrument Wash Buffer	500 ml Nuclease-free water	500 ml Nuclease-free water
2 Xenium Sample Wash Buffer A	1 L PBS-T	1 L PBS-T
3 Xenium Sample Wash Buffer B	150 ml Nuclease-free water	1 L PBS-T
4 Xenium Probe Removal Buffer	300 ml Probe Removal Buffer	500 ml Probe Removal Buffer

Reagent Plates

See assay-specific Reagent Preparation & Loading chapter for instructions on loading reagent bottles.

Pipette Tip Rack

a. Place a new pipette tip rack directly into the lower left position on the plate deck with the A1 tip position in the top-left corner.

b. Lower rack straight down and push down firmly on the bottom-right corner until you hear a click. Make sure it is sitting flat and level inside the plate deck. If it is slanted or not secure, remove and place again. Remove the pipette tip rack lid.

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

Pipette tip rack should align with Reagent Plates. Confirm after placing tip rack in place and adjust plates if necessary. Improper placement will result in a failed run.

Confirm Reagent Plates and Tip Rack Loading*

CORRECT
All reagent plates are level with tip rack

INCORRECT
Reagent Plate A is not level with tip rack (white arrow)

*Ensure tip rack is seated correctly for accurate comparison

Load Consumables *contd.*

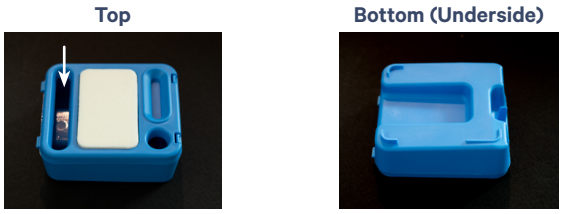
Extraction Tip

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.

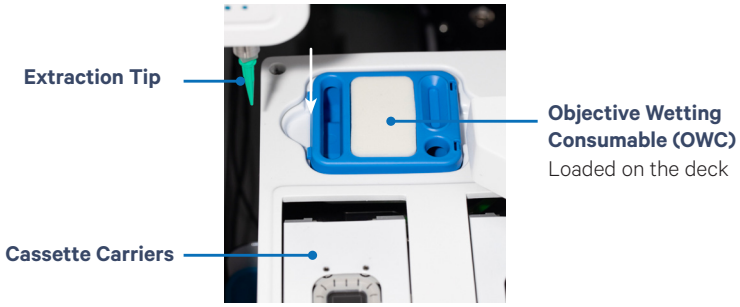
Objective Wetting Consumable (OWC)

a. Place a new OWC behind the cassette carrier with the reagent priming reservoir on the left.

Loading the Objective Wetting Consumable



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



Waste Tip Tray

a. Load empty waste tip tray into waste tip drawer and close drawer.

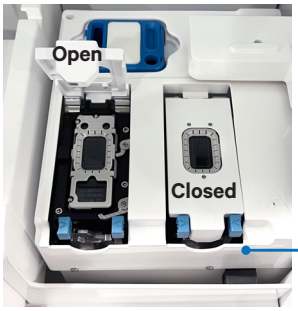
Load Consumables *contd.*

Slides from different Xenium workflows cannot be run together on the same instrument run.

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



Xenium Cassette shown

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: Use cotton swab to clean crevices if necessary.

Follow local lab safety or EHS requirements for using compressed air.

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 and slide is not cracked.

See the Troubleshooting section to fix leaking cassette assembly.

DO NOT proceed with run if slide is cracked or broken. Cracked or broken slides will result in instrument failure and replacement reagents will not be provided.

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.

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

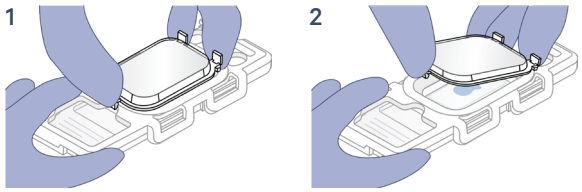
g. Confirm that the slide ID on the slide matches the ID number shown on the touchscreen.

Load Consumables *contd.*

h. Remove cassette lid. DO NOT spill or remove PBS-T covering the slide to ensure that the sections do not dry up. Save lid if storing postrun.

Xenium Cassette Lid Removal

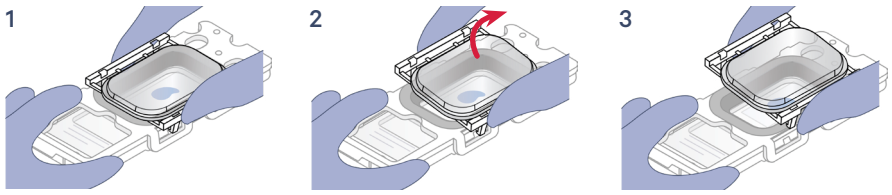
Xenium Cassette Lid *(CG000582 and CG000749)*



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.

Xenium Cassette Lid v2 *(CG000760)*



Apply even pressure on both sides of the lid using thumb and index finger.

While maintaining inward pressure, pull upward with index finger to unhook the side feet from the cassette.

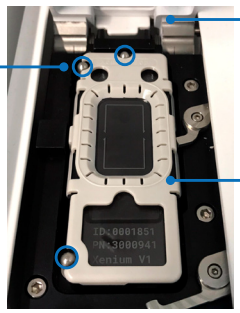
Slowly lift cassette lid up to unhook the remaining lid feet. Ensure no liquid splashes out of the well

i. Fully open the cassette carrier lid. Place the cassette into the carrier as shown below.

Ensure cassette is loaded correctly prior to closing carrier lid to avoid damage. DO NOT proceed with run if slide is cracked or broken. Cracked or broken slides will result in instrument failure and replacement reagents will not be provided.

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

Xenium Cassette shown

Load Consumables *contd.*

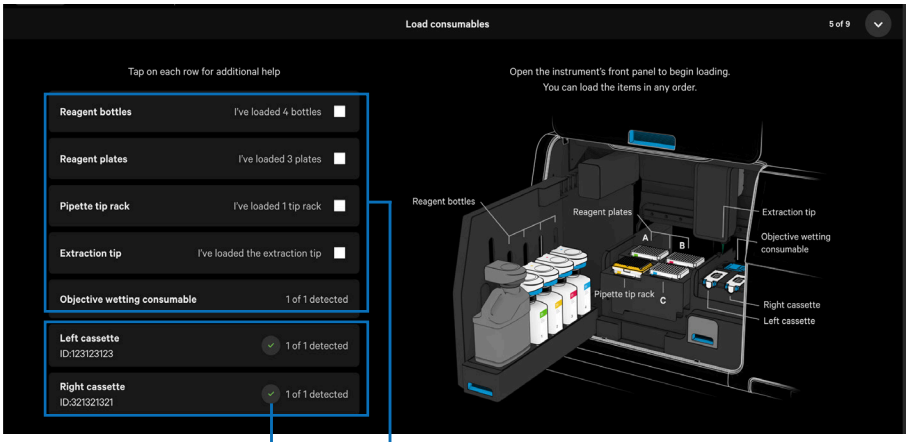
j. Close the cassette carrier lid until it clicks into place.

k. Repeat for the Right Cassette.

Confirm All Consumables Loaded

Consumables Detected

Actual screen may vary based on workflow selected.



Success

Green check indicates successful loading of specific consumable

User Confirmation

User must manually click check box to confirm specific consumable has been loaded

On the touchscreen, visually and manually confirm all consumables are loaded correctly and click "Continue". Close the instrument front panel.

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 2-6 days › Cleanup ~5 min › Unload ~10 min

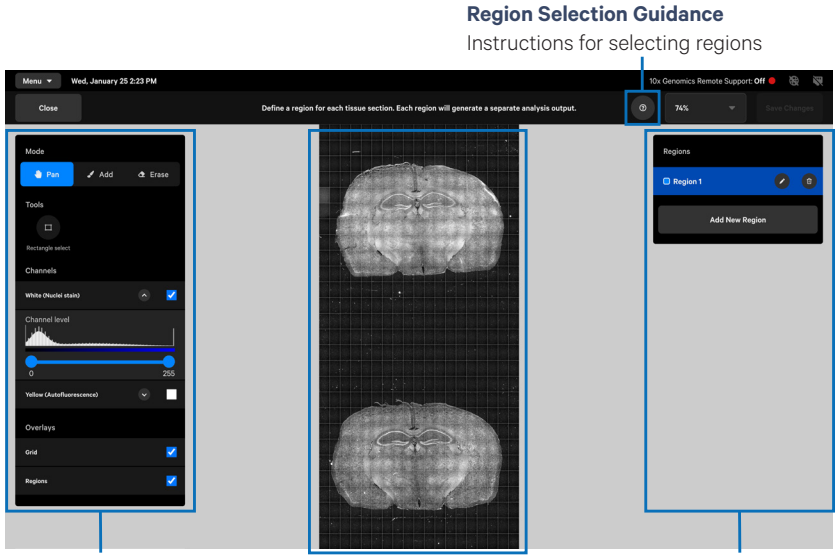
a. Instrument will begin Sample Scan. The scanned image will be used for region selection.

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, keyboard, and trackpad during the scan. The instrument front panel 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.

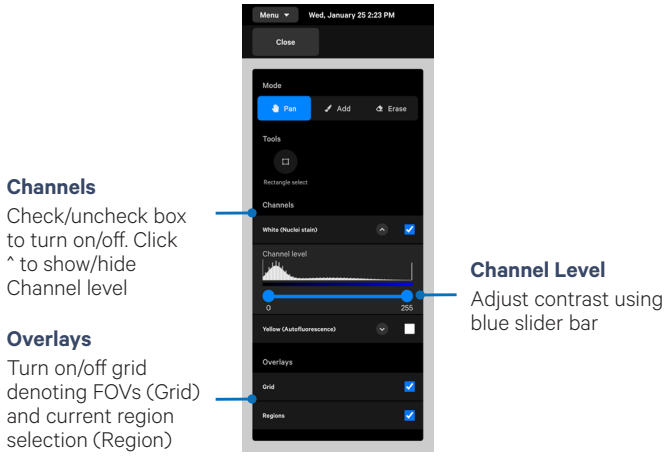
Overview Scan
Layout and features seen following completion



Sample Scan
contd.

c. Review sample scan image. Use the panel on the left side of the screen to turn on/off channels, adjust channel levels, and view overlays. Fine-tuning will help with region selection.

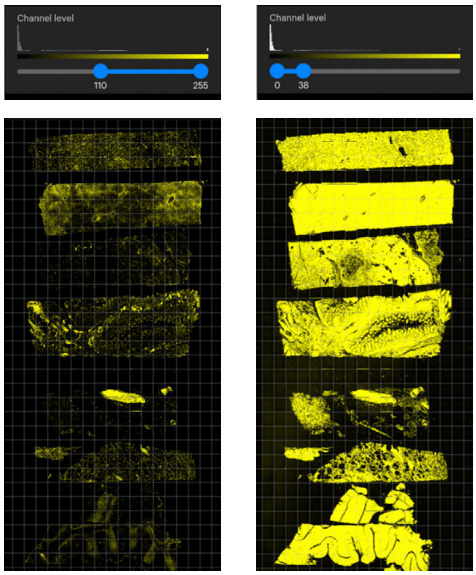
Overview Scan Image Options
Left panel on screen after overview scan is completed



TIPS

To define tissue morphology better 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 do not impact image acquisition during run.

Sample Scan
contd.

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.

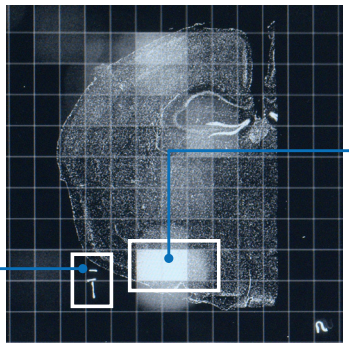
TIPS

High levels of autofluorescence in overview scan are 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.

Even if significant debris is visible, initiate the run. Contact support@10xgenomics.com if run assessment is needed.

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 likely be removed during the instrument run.

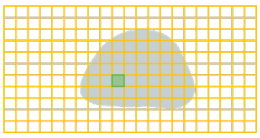
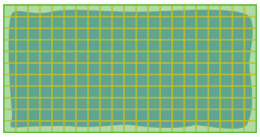
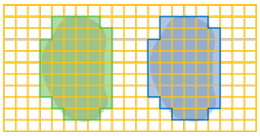
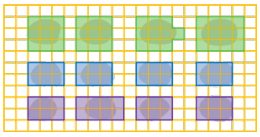
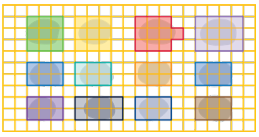
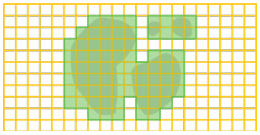
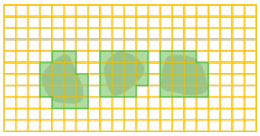
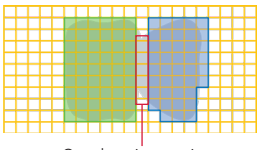
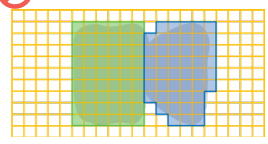
Region Selection

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
At least one region must be selected per slide. Region selection size: • Minimum: one FOV* • Maximum: entire well	Minimum size (one FOV)  Maximum size (entire well) 
User defines number of tissues in each region. *Subtissue section selection is not recommended*	
Consolidating regions is recommended for slides with multiple sections ***Deselect blank FOVs.*** *No more than 8 regions can be selected per slide*	✓ 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  Overlapping regions Do NOT select ⊘ Incorrect 

TIPS *Region names must be unique across all slides and 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

Initialize ~10–20 min > Load ~5 min > Sample Scan 1 h > Region Sel. ~10 min > **Run** 2-6 days > Cleanup ~5 min > Unload ~10 min

a. Confirm consumables loaded, run settings, and cassette details and 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, keyboard, and trackpad during the run.

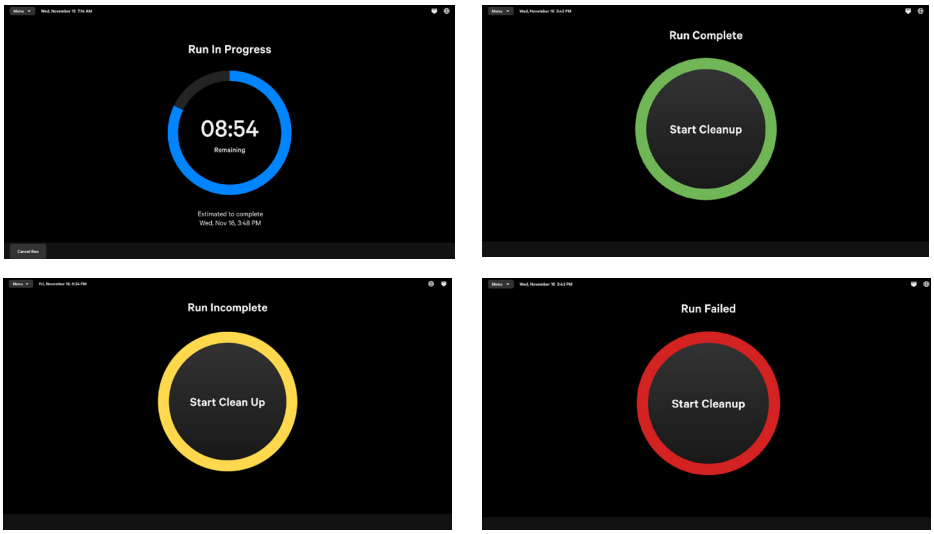
b. Touchscreen will display run progress and estimated time remaining. To cancel run at any time, click the "Cancel Run" button at the bottom-left corner of the screen. Run information is shown in the following colors:

Blue indicates run in progress.

Green indicates completed run.

Yellow indicates that the run is incomplete.

Red indicates that the run has failed.



See 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.

c. Registered users of a 10x Cloud-connected Xenium Analyzer can monitor instrument status and run progress on the cloud. Consult the Monitor Run Time Progress page for details.

Postrun Cleanup

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

Cleanup should 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 "Next" when complete.

Unload Consumables

Follow local and institutional guidelines for proper handling and disposal of volatile and hazardous chemicals and solid waste.

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

Follow local lab safety or EHS requirements for using compressed air.

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

Cassettes & Slides

a. Squeeze the release buttons 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. Postrun, remove the liquid covering the slide, and add **1,000 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 postrun H&E,*** *consult the Xenium In Situ Gene Expression - Post-Xenium Analyzer H&E Staining Demonstrated Protocol for Quencher Removal followed by H&E staining (CG000613).*

Waste Bottle *(Reusable)*

a. Slide the bottle carrier tray and remove the Waste Bottle.

b. Discard liquid following institution or local guidelines.

The waste includes potentially volatile and hazardous chemicals. Follow institutional 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 move upwards.

b. Remove bottles from carrier. Uncap and empty at the appropriate liquid waste disposal following institution or local guidelines.

Xenium Probe Removal Buffer (blue label, bottle position 4) includes potentially volatile and hazardous chemicals. Follow institutional or local guidelines for proper waste disposal.

c. Push the bottle carrier back into place.

Unload Consumables *contd.*

Objective Wetting Consumable (OWC)

a. Discard following institutional or local guidelines for proper waste disposal.

Reagent Plates

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

Condensation under reagent plates postrun may be visible and is normal. Following plate removal, dry the area if condensation has occurred.

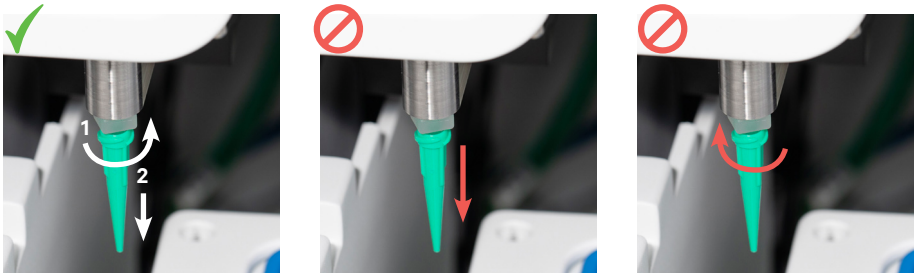
Pipette Tip Rack

a. Remove the tip rack and discard tips following institution or local guidelines for proper waste disposal.

Extraction Tip

a. Remove Extraction Tip as shown below.

Removing the Extraction Tip



Grip top part of tip, twist counterclockwise (1) and then pull down (2).

DO NOT pull down directly without twisting counterclockwise.

DO NOT twist clockwise.

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

Waste Tip Tray *(Reusable)*

a. Discard the used pipette tips from Waste Tip Tray following institutional or local guidelines for proper waste disposal.

b. 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”.

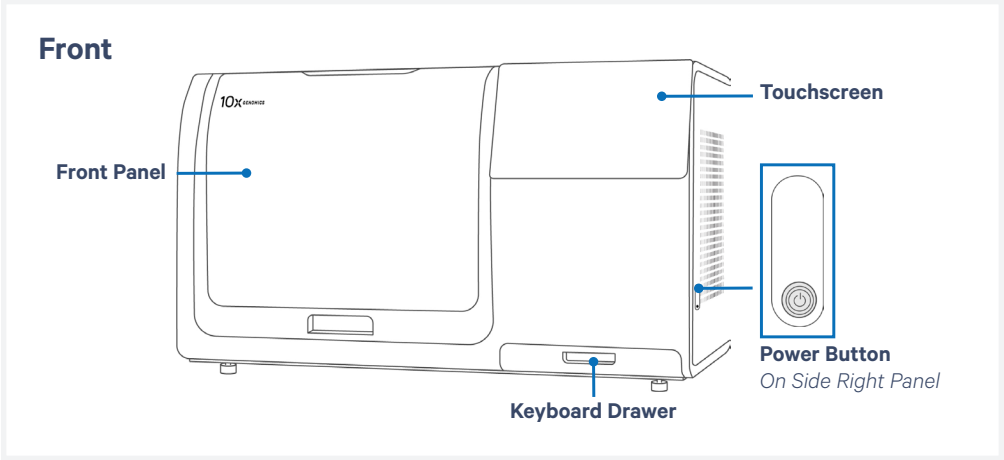
Powering OFF Instrument

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 (see 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. Do NOT use the touchscreen to shut down instrument.



10x GENOMICS

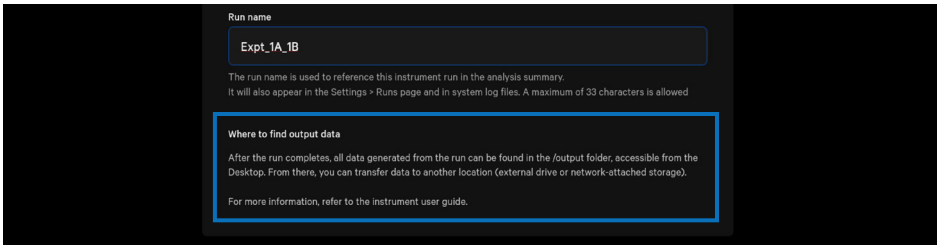
Data Output

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

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

Data Output
contd.

Option 1: Export to Network Shared Folder (Recommended Option)

Users can work with their institution's IT department to set up Local Area Network (LAN) for data transfers to a shared folder on a network-connected computer or device. Xenium Analyzer can be configured to work with nonpersistent networks such as Network File Share (NFS) or Common Internet File System (CIFS).

a. Click Menu and Open Settings. Select Runs on left-hand side and select click Export Run Data

b. Select the network drive desired. To set up a new network shared folder, click "Set Up" button located in the Set up a new network shared folder box.

c. Fill out the required information and click Connect when finished. If the connection is successful, the shared folder will appear as destination

d. Select the appropriate network shared folder and follow on-screen instructions to export run(s).

Option 2: Portable USB drive (Alternative Option)

a. Attach USB drive to the USB port on the Xenium Analysis Computer. USB drive must have ≥ 256 GB storage capacity, version 3.0 or higher, and be preformatted 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

b. Click Menu and Open Settings. Select Runs on left-hand side and select click Export Run Data

c. Select the USB drive desired. To connect a new USB, click "Check for USBs" located in the Connect a USB drive box. If the connection is successful, USB drive will appear as destination.

d. Select the appropriate USB drive and follow on-screen instructions to export run(s).

e. Once export is complete, it is safe to remove the USB drive.

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**.

Follow local lab safety or EHS requirements for using compressed air.

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 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.

Follow local lab safety or EHS requirements for using compressed air.

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

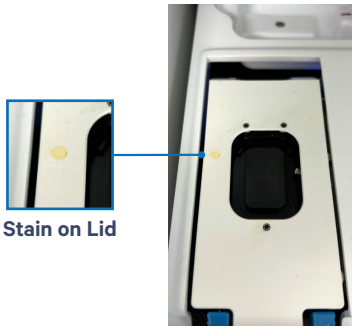
A dry, clean, lint-free surface on both the slide bottom and instrument cassette carrier is critical for a proper instrument run. Any debris or lint can interfere with image acquisition.

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.

Consult the 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.

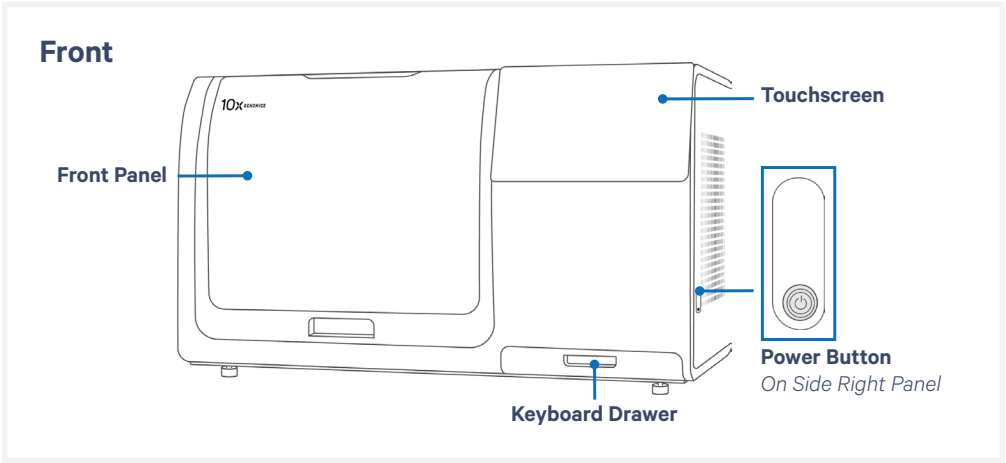
Powering OFF instrument

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

Do NOT power 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 shut down the instrument.



Service

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 nonapproved power cord as it may be inadequately rated to handle the electrical loads.

Environmental Requirements

It is the design intent of the Xenium Analyzer that it is 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, check that no liquid is leaking from the assembly. 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).

Gap after Cassette Assembly

Cassette assembly with a gap will result in reagent leakage. If gap is visible, disassemble and reassemble cassette before proceeding.

- For Xenium Cassette v2, slide is not placed underneath slide clip.
- Assembly is placed on a dry surface. If 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. See Xenium Cassette Quick Reference Card (Document CG000623) for disassembly and assembly instructions.

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.

If leak persists, slide may be cracked. DO NOT proceed with instrument run. Cracked or broken slides will result in instrument failure and replacement reagents will not be provided.

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

Troubleshooting
contd.

Storing slides after instrument failure

In the event of run failure, slides may be stored for a future run. Cassettes should always be stored hydrated with the recommended reagent and stored at the recommended temperature to maintain sample integrity.

Short-term Storage (≤ 1 week), All assays:

a. Store in 1,000 µl PBS-T at 4°C in the dark. Ensure that the slide is stored in microbe-free and nuclease-free conditions, with a Xenium Cassette Lid applied to prevent evaporation.

Long-term Storage

Xenium v1 assays: 1 week – 2 months
Xenium Prime and Xenium Protein assays: 1 week - 1 month

a. Remove all PBS-T from the cassette well.

b. Add **1,000 µl** 70% ethanol, incubate for **2 min** at **room temperature**, remove the ethanol.

c. Add **1,000 µl** 100% ethanol, incubate for **2 min** at r**oom temperature**, remove the ethanol.

d. Add **1,000 µl** 100% ethanol, incubate for **2 min** at **room temperature**, remove the ethanol.

e. Remove slide from the cassette and place in a slide mailer containing 10 ml cryoprotectant or more to fully submerge the slide (30% Glycerol prepared in PBS is recommended).

f. Clean the cassette. Discard lid.

i. Rinse under running Milli-Q water

ii. Spray with 70% isopropanol

iii. Repeat Milli-Q water and 70% isopropanol wash

iv. Rinse under running Milli-Q water

v. Air dry and save for a subsequent instrument run.

f. Store at -20°C for up to **2 months**.

g. When ready to use:

i. Equilibrate the mailer with the slide to **room temperature**

ii. Once completely thawed, rinse the mailer 3X with **10 ml** PBS-T

iii. Remove the slide from the mailer and assemble in the cassette

iv. Add **1,000 µl** PBS-T to the cassette well.

Contact support@10xgenomics.com if more information is needed.

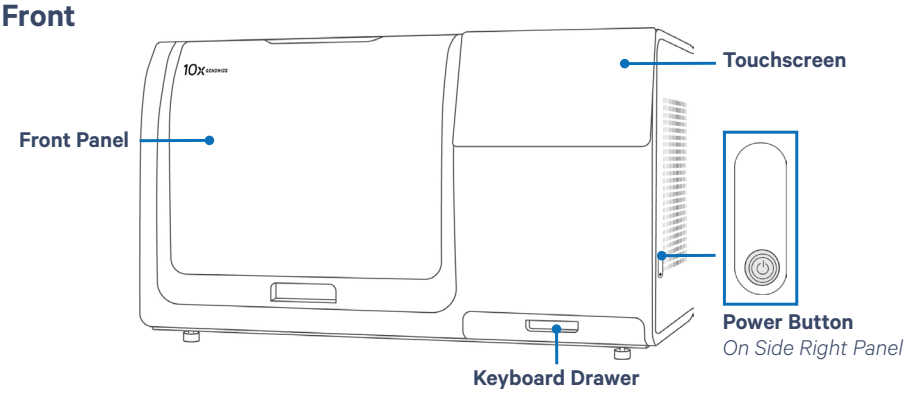
Troubleshooting
contd.

Full Instrument Shutdown

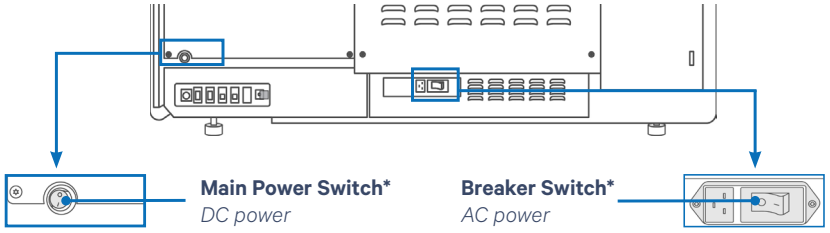
In some instances, it may be necessary to perform a full power-cycle of the instrument when instructed by 10x Genomics personnel.

Contact support@10xgenomics.com prior to attempting the following steps.

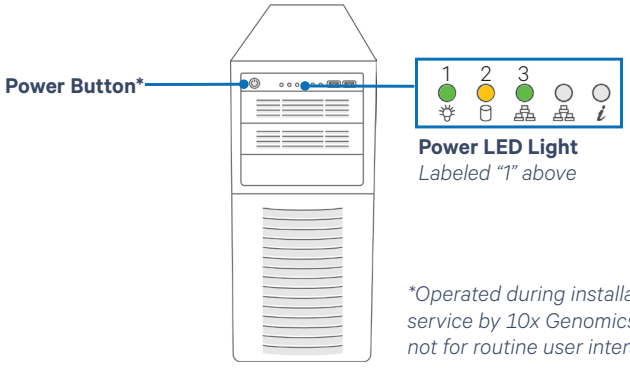
Front



Back



Xenium Analysis Computer Front



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

Troubleshooting
contd.

a. Press and hold the side power button down for **3 sec**. The touchscreen monitor should turn black.

b. Wait at least **3 min** for the internal computer and Xenium Analysis Computer to power down.

Ensure that the Power LED on the Xenium Analysis Computer is OFF before proceeding to the next step.

*If the Xenium Analysis Computer does not shut down after **>10 min**, manually shut down it by holding down the red Power Button for **3 sec**.*

c. Toggle the black Main Power Switch to the OFF position.

d. Toggle the white Breaker Switch to the OFF position. Wait **3 min**.

e. To power the system back ON, toggle the white Breaker Switch to the ON position.

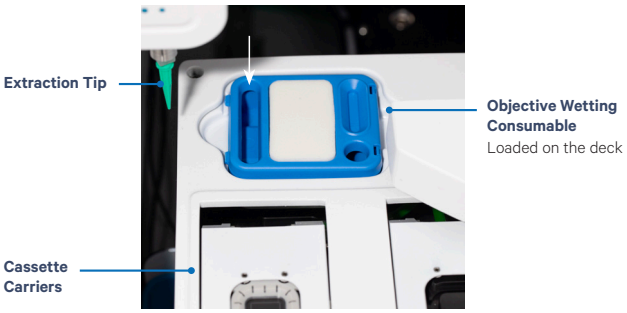
f. Toggle the black Main Power Switch to the ON position

g. Turn on the Xenium Analysis Computer by pressing the red Power Button for **3 sec** or until the Power LED illuminates.

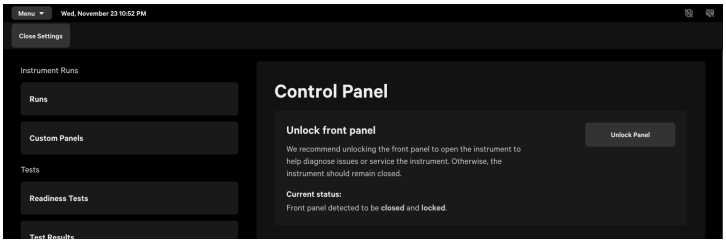
Troubleshooting
contd.

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	Apply even pressure to push front panel in. No gap should be visible from side angle.
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. At least one cassette carrier must be loaded.
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.

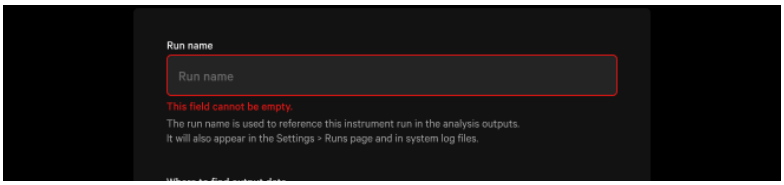
Error	Solution
During loading and unloading consumables	
Need to open the front panel to access the instrument deck prior to starting a run	Unlock the front panel from Menu Settings, accessible on the top bar of the screen. 
During data analysis	
Insufficient storage available	There is insufficient storage 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. See 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. See 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.
USB not ejecting properly	Minimize application and open Files on Desktop. Right-click USB in the sidebar and select Safely Remove Device or Unmount.
Other	
Screen/instrument frozen	Contact support@10xgenomics.com for assistance before proceeding.

Errors

Errors can appear in different ways on the instrument. On-screen instructions will guide the user through recoverable errors. If the error continues, contact support@10xgenomics.com with the error code.

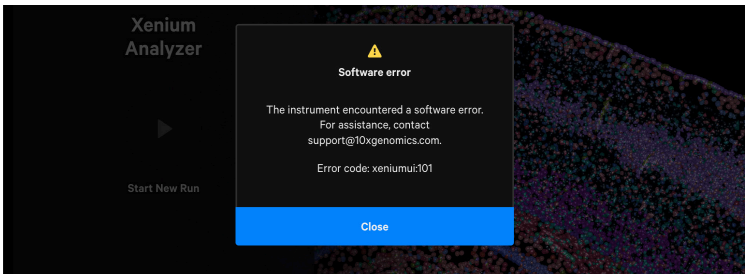
Contextual Error Messages

While completing information fields, invalid input is noted by a red bounding box. Guidance will appear adjacent to the input field.



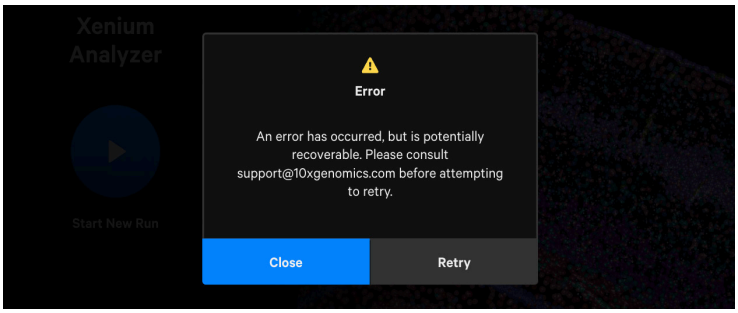
Error Alerts

Pop-up error alerts may be seen. Follow on-screen instructions.



System Retry

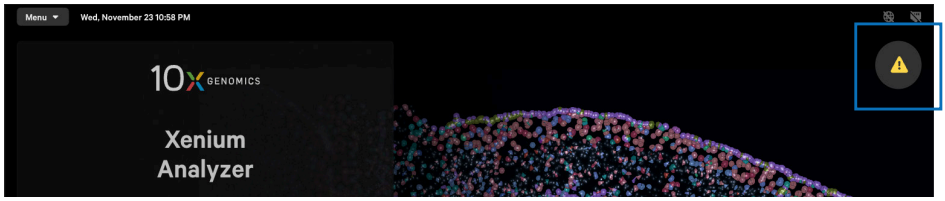
Some errors may provide the option to retry the previous system operation. Email support@10xgenomics.com for assistance before attempting retries.



Errors
contd.

Home Screen Error Indicator

Some errors prevent the user from starting a new run. Click the button at the upper right corner of home screen and follow on-screen instructions.



Critical Errors

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

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.

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