91600131 Rev C

MERSCOPE Ultra™

Instrument User Guide

vizgen®

NOTICES

Document number	91600131
Document revision	Rev C
Revision date	September 2025

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WARNINGS

Vizgen laser safety is evaluated according to the IEC 60825 1:2014 and found to be conforming to the class 1M. This device complies with 21 CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No.50, dated June 24, 2007 and Laser Notice 56, dated 1/19/2018.

Viewing the laser output with telescopic optical instruments, such as binoculars or telescopes, may pose an eye hazard and thus the user should not direct the beam to an area where such instruments are likely to be used.

Electrical Safety: *Conforms to UL STD 61010-1 Certified to CSA STD C22-2# 61010-1-12 Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General Requirements*

User should exercise caution in attaching and removing electrical cables only when power is removed from the MERSCOPE Ultra Instrument (power is de-energized). The improper use of these cables can result in the potential of electric shock.

For the safety of the user:

- User shall use the Vizgen-supplied main power cable. The use of any other main power cables can result in the malfunction of the instrument and can result in the potential of electrical shock to the users.
- Only authorized Vizgen representatives should uncrate and install the MERSCOPE Ultra Instrument. Mishandling of the instrument can affect the alignment or damage instrument components.
- Do not relocate the instrument after installation and preparation. Moving the instrument improperly can affect optical alignment and compromise data integrity. If the instrument must be relocated, contact Vizgen Support (support@vizgen.com).
- Uncrating or moving an instrument by anyone other than an authorized Vizgen representative will void the warranty.
- Never use an extension cord to connect the instrument to a power supply.

SUPPORT

Contact information

support@vizgen.com
61 Moulton Street
West Cambridge Science Park
Cambridge, MA 02138
USA

Other Vizgen references are available online at https://vizgen.com/

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1 INTRODUCTION

1.1 The MERSCOPE Ultra Workflow

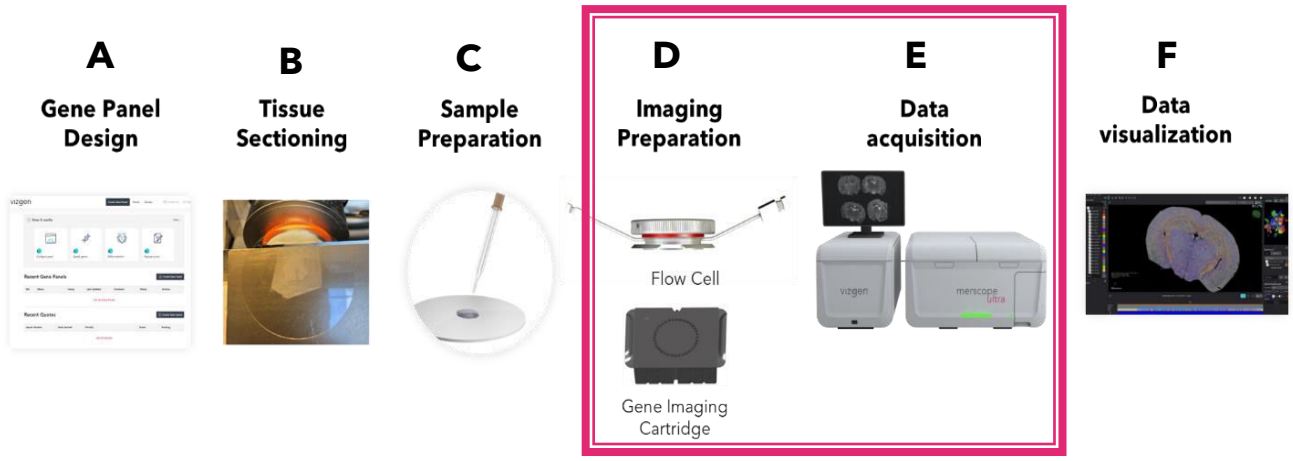


Figure 1. Workflow steps in the MERSCOPE Ultra Platform. **A)** Gene panel design. **B)** Tissue sectioning. **C)** Sample preparation. **D)** Imaging preparation. **E)** Data acquisition. **F)** Data visualization.

1.2 The Vizgen MERSCOPE Platform Solution

The Vizgen MERSCOPE Ultra Platform provides an end-to-end solution for the implementing of the MERFISH technique for spatial transcriptomics, from sample preparation to data analysis and visualization (Figure 1). The first step in any project is to select your MERFISH gene panel using Gene Panel Portal (Figure 1A). When you are ready to run your experiment, the tissue is first sectioned onto a MERSCOPE Slide (Figure 1B). Sample Preparation prepares the section for imaging on the MERSCOPE Ultra, which is covered in detail in this document (Figure 1C). Once the Slide is prepared, the MERSCOPE Ultra flow chamber and Gene Imaging Cartridge are assembled and activated (Figure 1D), then analysis is performed on the MERSCOPE Ultra Instrument (Figure 1E). Analysis is performed on the MERSCOPE Ultra Analysis Computer) and further exploration of the data is done through the MERSCOPE Vizualizer software (Figure 1F).

1.3 The MERSCOPE Workflow

Vizgen offers two MERSCOPE instruments: MERSCOPE and MERSCOPE Ultra. This User Guide covers the preparation, operation, and analysis of samples on the MERSCOPE Ultra instrument.

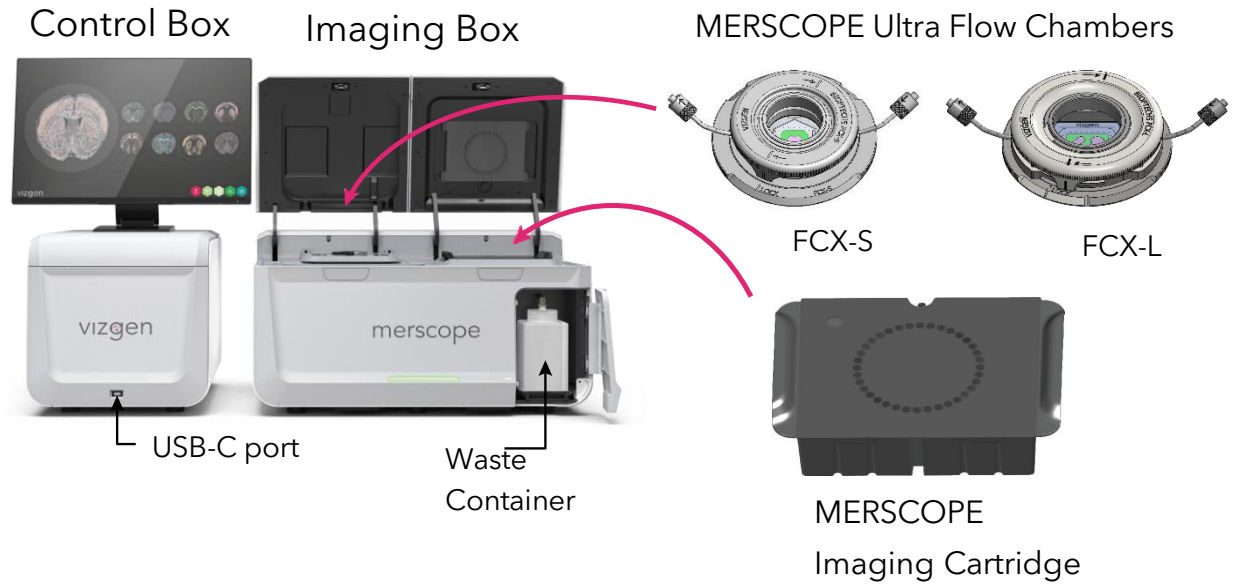
The MERSCOPE Ultra User Guide starts **after** completion of Sample Preparation. Please refer to the appropriate documents for the preceding steps of the workflow:

Step	User Guide	PN
Histological sectioning (FFPE)	MERSCOPE Tissue Preparation Guide: Histology Guide for Preparing FFPE Samples for Experiments on the MERSCOPE® Platform	91600126
Histological sectioning (Fresh or Fixed Frozen)	MERSCOPE Tissue Preparation Guide: Histology Guide for Preparing Fresh and Fixed Frozen Tissue Samples for Experiments on the MERSCOPE Platform	91600129
Sample Preparation (MERFISH 2.0)	MERFISH 2.0 Sample Preparation User Guide for Sectioned Tissue Samples	91600132
Sample Preparation (FFPE; MERFISH 1.0)	MERSCOPE FFPE Tissue Sample Preparation User Guide	91600112
Sample Preparation (Fresh or fixed frozen; MERFISH 1.0)	MERSCOPE Fresh and Fixed Frozen Tissue Sample Preparation User Guide	91600002

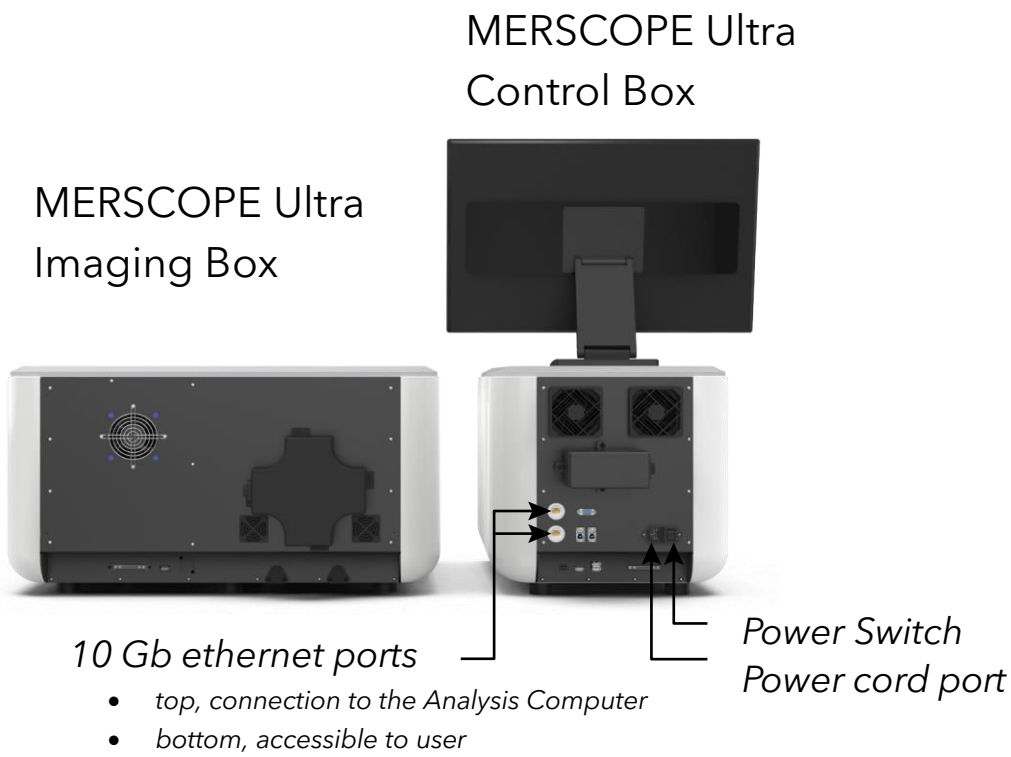
Load and run the MERSCOPE Ultra Instrument. The MERSCOPE Slides (40mm round slide and 47x57mm rectangular slide) are assembled into the MERSCOPE Flow Chambers, FCX-S (Standard) and FCX-L (Large) respectively, and then loaded into the instrument along with a MERSCOPE Imaging Cartridge. Users define regions of interest on the MERSCOPE Slide within the system software and initiate the fully automated instrument run.

Data Processing and Visualization. The MERSCOPE Ultra Instrument Software (in combination with the MERSCOPE Ultra Analysis Computer) automatically processes the raw images to output spatial genomics measurements in a format ready for immediate downstream analysis. The output includes the list of all detected transcripts and their spatial locations in three dimensions (CSV files), mosaic images (TIFF), experiment metadata (JSON), output from the cell segmentation analysis: transcripts per cell matrix (CSV), cell metadata (CSV), cell boundaries (PARQUET), and a binary for use with the MERSCOPE Vizualizer software. The MERSCOPE Ultra Platform includes the MERSCOPE Vizualizer software for visualizing and analyzing data. The output files are also compatible with open-source tools for single-cell and spatial analysis.

2 MERSCOPE ULTRA INSTRUMENT OVERVIEW



MERSCOPE Flow Chamber in the MERSCOPE Ultra Imaging Box	MERSCOPE Imaging Cartridge in the MERSCOPE Ultra Imaging Box
	



- *top, connection to the Analysis Computer*
- *bottom, accessible to user*

The MERSCOPE Ultra Instrument is a combination of the MERSCOPE Ultra Imaging Box and MERSCOPE Ultra Control Box, where the instrument is programmed via an intuitive touchscreen user interface. The MERSCOPE Ultra Analysis Computer processes the images acquired on the instrument to generate the list of detected transcripts, mosaic images, and cell segmentation results.

If the instrument is not connected to the internet, the applicable MERSCOPE Codebook must be transferred to the instrument in advance of initiating an experiment. The intuitive instrument user interface guides users through experimental setup.

The MERSCOPE imaging cartridge is supplied and stored at −20°C and must be fully thawed before use. When thawed, the imaging cartridge is activated by manual addition of an activation mix, followed by layering with mineral oil. The activated imaging cartridge is then inserted into the instrument.

The MERSCOPE Slide from sample preparation undergoes final preparation on the bench before careful assembly into the MERSCOPE Flow Chamber. The optical surface is cleaned, and it is connected to the instrument fluidic lines.

The MERSCOPE Ultra Instrument first acquires a low-resolution mosaic. Then, users select the regions of interest for MERFISH imaging, switch the instrument to a high-magnification objective, and start the fully-automated experiment.

3 INSTRUMENT PACKING LISTS

3.1 Vizgen Materials

MERSCOPE Ultra Instrument	**10000108**
MERSCOPE Ultra Imaging Box	10100110
MERSCOPE Ultra Control Box	10100112
MERSCOPE Ultra Monitor	61500001
MERSCOPE Ultra Wireless Keyboard and Mouse	61500008
MERSCOPE Ultra Analysis Computer	10200100
MERSCOPE Ultra Instrument Waste Container	10300002
MERSCOPE Ultra Instrument Power Cords (regional)	Regional
MERSCOPE Ultra Instrument Connections	-
- Ethernet cable	61500004
- Fiber-optic cable	61000125
- USB cables (2x)	60400129
- Communication/interbox power entry cable	10700124
MERSCOPE Ultra Standard Flow Chamber (FCX-S)	10300106
MERSCOPE Ultra FCX-S Flow Chamber Gasket	10300114
MERSCOPE Ultra Large Flow Chamber (FCX-L)	10300105
MERSCOPE Ultra FCX-L Flow Chamber Gasket	10300115
MERSCOPE Wash Cartridge	10700102

MERSCOPE Instrument Accessory Kit	**20100005**
Imaging Fluid Line Adapter (for verification experiments) (5x)§	30400010
Fluidic Line Connector (for fluidic line wash/preparation for idling) (2x)§	60900123
Immersion Oil†	30400007
Hobby Blade Handle*	30400005
Hobby Blades (pack)*	30400012
High Precision Tweezers*	30400004
Serrated Tweezers*	30400003
Lens Cleaning Tissue§	30400006
Luer-lock Syringe (5x)§	30400011
25x25 mm^2 Large Gel Coverslip	10500130
20 mm Diameter Gel Coverslip	30200004
Flow Chamber Torque Adapter	60900130

†The safety data sheet for immersion oil may be obtained from Vizgen Support (support@vizgen.com).

*For use in sample preparation. If additional parts are needed, refer to sample preparation user guides for ordering information, available online at https://vizgen.com/.

§The recommended supplier and part number for additional materials is provided in Required Materials and Recommended Suppliers.

4 IMAGING CONSUMABLES

4.1 Vizgen Materials

Use Sample Prep Wash Buffer (PN 20300001) and Formamide Wash Buffer (PN 20300002) from the applicable MERSCOPE sample preparation kit. All Gene Imaging Kits include one gene imaging cartridge with required Imaging Buffer Activator and DAPI/Poly Staining Reagent. Gene Imaging Kits are specific to the MERFISH workflow used in sample preparation (MERFISH 1.0 or MERFISH 2.0) and are needed for the FCX-S (Standard) and FCX-L (Large) flow chambers.

MERSCOPE Ultra Flow Chamber	MERSCOPE Ultra Gene Imaging Kits	Storage	Item
FCX-S (Standard)	MERSCOPE Standard 140 Gene Imaging Kit	-20°C	10400004
	MERSCOPE Standard 140 Gene Imaging Kit V 2.0	-20°C	10400167
	MERSCOPE Standard 300 Gene Imaging Kit	-20°C	10400005
	MERSCOPE Standard 300 Gene Imaging Kit V 2.0	-20°C	10400168
	MERSCOPE Standard 500 Gene Imaging Kit	-20°C	10400006
	MERSCOPE Standard 500 Gene Imaging Kit V 2.0	-20°C	10400169
	MERSCOPE Standard 1000 Gene Imaging Kit	-20°C	10400126
	MERSCOPE Standard 1000 Gene Imaging Kit V 2.0	-20°C	10400170
FCX-L (Large)	MERSCOPE Large 140 Gene Imaging Kit	-20°C	10400163
	MERSCOPE Large 140 Gene Imaging Kit V 2.0	-20°C	10400171
	MERSCOPE Large 300 Gene Imaging Kit	-20°C	10400164
	MERSCOPE Large 300 Gene Imaging Kit V 2.0	-20°C	10400172
	MERSCOPE Large 500 Gene Imaging Kit	-20°C	10400165
	MERSCOPE Large 500 Gene Imaging Kit V 2.0	-20°C	10400173
	MERSCOPE Large 1000 Gene Imaging Kit	-20°C	10400166
	MERSCOPE Large 1000 Gene Imaging Kit V 2.0	-20°C	10400174

Safety Data Sheets are available online at https://vizgen.com/

4.2 Required Materials and Recommended Suppliers

Item	Vendor	Part number
Buffers and additives		
Ethyl Alcohol, Pure (200 proof)	Millipore-Sigma	E7023-6X500ML
RNase Inhibitor, Murine	NEB	M0314L
Solutions and consumables		
Fluidic Line Connector (for fluidic line wash/preparation for idling)	McMaster-Carr	7033T21
Mineral Oil	Millipore-Sigma	M5904-6X500ML
RNaseZap RNase Decontamination Solution	Thermo Fisher	AM9782
Lens Tissue	Thorlabs	MC-5
Serological Pipet (25 mL)	VWR	82051-182
Cleaning tissue (Kimwipe or similar)	VWR*	21913-214*
Imaging Fluid Line Adapter (for verification experiments)	VWR	45508-22
Luer-lock Syringe	VWR	76290-380
1000-µL and 200-µL pipette and compatible tips	-	-
Gel coverslip, 20 mm diameter, round	Fisher	NC0308916
Gel coverslip, 25X25 mm, square	Corning	CLS285525-100EA
*Alternative to Kimwipe.		

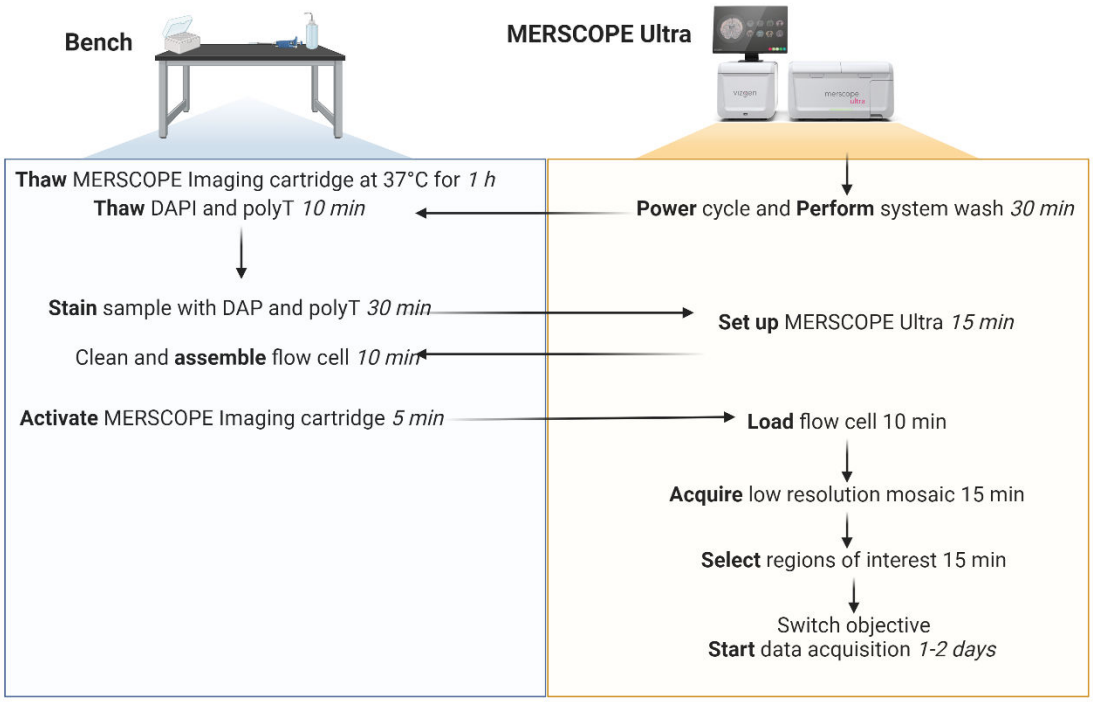
4.3 Laboratory Setup

General laboratory equipment required (in addition to equipment used for sample preparation). General laboratory equipment should be used per manufacturer's instructions.

Item	Vendor	Part number
Water bath	VWR	76308-896

An alternative water bath may be used if it is large enough to accommodate the MERSCOPE Imaging Cartridges: 8 × 11 in (20 × 28 cm).

5 MERSCOPE ULTRA IMAGING OVERVIEW



6 MERSCOPE ULTRA INSTRUMENT QUICK REFERENCE GUIDE

Step	Summary
From Sample Preparation	1. Sample is on a MERSCOPE Slide in a solution/buffer
Power Cycle	1. Power cycle the system by: a. Shutting down the Instrument Computer and turning off the Control Box b. Waiting 60 seconds c. Powering on the Control Box 2. Double click the MERSCOPE application icon to open the instrument user interface.
Confirm Storage Space	1. Click Settings (gear icon) on the home page top navigation bar to inspect storage capacity. 2. Confirm there is at least 7.5 TB of disk space available on the Instrument Computer. 3. Confirm at least 2 TB of disk space is available on the Analysis Computer. 4. If insufficient space is available on either device, old datasets should be copied to network storage or a portable USB hard drive.
Wash Instrument	1. Click Maintenance on the home page top navigation bar. 2. Click **Start instrument wash** a nd follow the onscreen prompts to perform the Instrument Wash Cycle.
Power Cycle	1. Power cycle the system by: a. Shutting down the Instrument Computer and turning off the Control Box b. Waiting 60 seconds c. Powering on the Control Box 2. Double click the MERSCOPE application icon to open the instrument user interface.
Begin Experiment Configuration	1. Start MERFISH in the instrument user interface. Configure the instrument with experiment configuration and sample details. 2. Specify the panel-specific MERSCOPE Codebook. 3. Pause in control software and proceed to thaw cartridge and stain sample. **Ensure the codebook matches the experimental workflow (MERFISH 1.0 or MERFISH 2.0)**
Thaw Cartridge	1. Start thawing of applicable MERSCOPE Imaging Cartridge in the 37°C water bath for 60 min. **Ensure the cartridge matches the experimental workflow (MERFISH 1.0 or MERFISH 2.0) and slide size (Standard or Large)** **DO NOT allow the valve to come into contact with the water.**

Stain Sample	1. On the bench, stain the sample on a MERSCOPE Slide with DAPI and PolyT stain. **Ensure the Staining Reagent matches the experimental workflow (MERFISH 1.0 or MERFISH 2.0)**
Load Cartridge and **Continue** Experiment Configuration	1. Activate a thawed MERSCOPE Imaging Cartridge by manual addition of Imaging Activation Mix and a layer of mineral oil. Ensure cartridge size (Standard or Large) aligns with the flow chamber selected for the experiment: FCX-S (Standard) or FCX-L (Large), respectively. 2. Insert the activated imaging cartridge and prime the fluidic lines.
Load Flow Chamber	1. Assemble the stained MERSCOPE Slide into the MERSCOPE Ultra Flow Chamber. 2. Connect the assembled flow chamber to the instrument fluidic lines when prompted. 3. Wet the flow chamber and ensure there are no air bubbles. 4. Insert the wetted flow chamber into the instrument and lock into place.
Select Regions of Interest	1. Acquire a low-resolution mosaic (fully automated) and define regions of interest – up to 10 regions can be selected with a total area of up to 125 mm^2 for FCX-S (Standard) and 300 mm^2 for FCX-L (Large).
Switch Objective	1. Remove the assembled MERSCOPE Flow Chamber from the instrument WITHOUT detaching the fluidic lines. 2. Apply immersion oil to the high-magnification objective. 3. Re-insert the flow chamber into the instrument and lock into place.
Data Acquisition	1. Acknowledge high magnification was successful and acquire sample data (fully automated). 2. After the experiment is complete, select Segmentation Parameters unless pre-selected during experiment setup. 3. After segmentation parameters are selected, review the selection, and initiate image processing analysis.
Clean	1. Empty the waste container, remove the MERSCOPE Flow Chamber from the instrument and clean the immersion oil from the high-magnification objective. Install a Fluidic Line Connector. 2. Disassemble and clean the MERSCOPE Flow Chamber. Remove and dispose of the MERSCOPE Imaging Cartridge.IF the instrument will idle or only run verification for ≥2 weeks, follow the idle period preparation procedure.
Power Cycle	1. Power cycle the system by: a. Shutting down the Instrument Computer and turning off the Control Box b. Waiting 60 seconds c. Powering on the Control Box

	2. Double click the MERSCOPE application icon to open the instrument user interface.
Wash Instrument	1. Click Maintenance on the home page top navigation bar. 2. Click **Start instrument wash** and click **Next** to advance through screens as operations are performed.
Transfer Data	1. Data may be transferred off the instrument from Z:\ merfish_output after image processing is complete (via mapped network drive or portable hard drive).

7 MERSCOPE ULTRA INSTRUMENT IMAGING STEP-BY-STEP

Sample preparation user guides instruct users on sample preparation through readiness for imaging. Prepared samples may be stored in a sealed petri dish in Clearing Solution/Clearing Premix at 37°C. Refer to the applicable sample preparation for specific instructions, available online at https://vizgen.com/. When ready, prepare for imaging and then proceed to DAPI and PolyT staining on the bench.

The cartridge, staining reagent, and codebook are specific to the chemistry used in your experiment. Carefully check that all materials align with the chosen chemistry (MERFISH 1.0 or MERFISH 2.0)

In this user guide, the term Slide refers to any MERSCOPE Slide used in sample preparation. The term "Cartridge" refers to any Gene Imaging Cartridge used in the protocol, and the term "Staining Reagent" refers to any DAPI and PolyT Staining reagent used in the protocol.

Symbol	Description
	Multiple options to proceed; depends upon experimental setup
	Note timing
	Overnight incubation or stopping point
	Visually inspect samples before proceeding
	Tip
	Critical step – follow instructions carefully

7.1 Prepare for Imaging: Power Cycle

1. If not already performed earlier in the day, power cycle the system by:
   a. Shutting down the Instrument Computer and turn the toggle switch on the back of the Control Box to the "Off" position.

b. Waiting 60 seconds
c. Switch the toggle switch on the back of the Control Box into the "On" position.

***IF** the instrument was prepared for an idle period using the MERSCOPE Ultra Instrument Idle Procedure, follow the MERSCOPE Ultra Instrument Restart After Idle Period procedure to prepare the instrument for imaging.*

***IF** the instrument has been idle for ≥2 weeks (or only verification runs during that time) and was **NOT** prepared using the MERSCOPE Ultra Instrument Idle Procedure, contact Vizgen Support (support@vizgen.com) for guidance.*

2. Double click the MERSCOPE application icon to open the instrument user interface.

Note: The data compression service takes a few minutes to initialize at startup and will show an error state for the Compression service on the Home Page Status Bar until initialization is complete.

7.2 Prepare for Imaging: Confirm Storage Space

1. Click Settings (gear icon) on the home page top navigation bar to inspect storage capacity. Confirm there is at least 7.5 TB of disk space available on the Instrument Computer.
2. Confirm at least 2 TB of disk space is available on the Analysis Computer.

The amount of data generated for an experiment will vary based on the experiment configuration. If disk space falls below 2 TB on the Analysis Computer during transfer, the transfer will stall until additional space is made available.

3. If insufficient space is available on either device, old datasets should be copied to network storage or a portable USB hard drive.

7.3 Prepare for Imaging: Wash Instrument

1. Click Maintenance on the home page top navigation bar.
2. Perform an Instrument Wash:

a. Click **Start instrument wash** and click **Next** to advance through screens as operations are performed. Details on the full procedure can be found in the INSTRUMENT WASH (MAINTENANCE) section.

7.4 Prepare for Imaging: Power Cycle the Instrument

1. Power cycle the system by:
   a. Shutting down the Instrument Computer and turn the toggle switch on the back of the Control Box to the "Off" position.
   b. Waiting 60 seconds
   c. Switch the toggle switch on the back of the Control Box into the "On" position.
2. Double click the MERSCOPE application icon to open the instrument user interface.

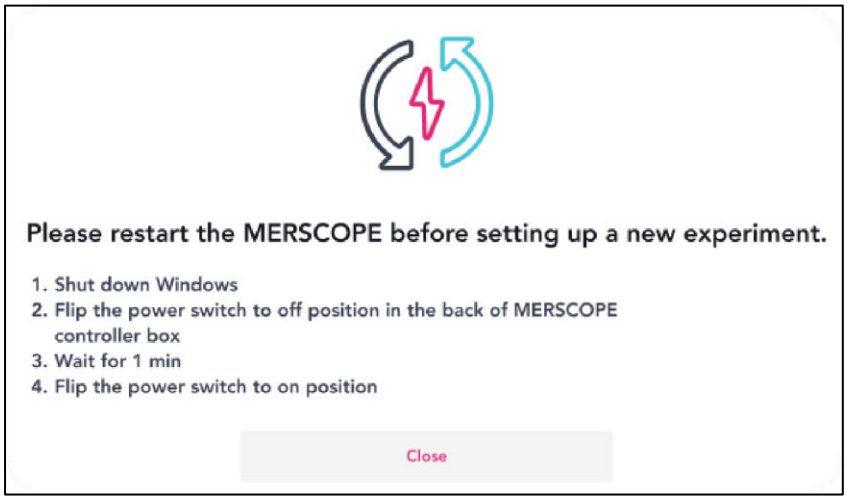
NOTE: The data compression service takes a few minutes to initialize at startup and will show an error state for the Compression service on the Home Page Status Bar until initialization is complete.

7.5 Begin Experiment Configuration

NOTE: To run MERFISH 2.0 chemistry, software version 234b or later versions are required. Please make sure MERSCOPE has the compatible software version for running the processed samples.

1. Click **Start MERFISH** on the display and allow 1-2 min for the instrument to initialize.
    a. *Prior to starting a new MERFISH experiment, there must be at least 7.5 TB of disk space available on the MERSCOPE Ultra Instrument Computer and 2 TB of disk space available on the MERSCOPE Ultra Analysis Computer. If insufficient space is available, old datasets should be copied to network storage or a portable USB hard drive.*

IF** the MERSCOPE has not been power cycled a prompt will display with instructions to power cycle the instrument. **The MERSCOPE must be power cycled before setting up a new experiment.



2. Select the applicable panel-specific MERSCOPE Codebook (may be imported from local storage).

NOTE: Check the codebook matches the gene panel used in the experiment (MERFISH 1.0 or MERFISH 2.0)

3. Pause here in the control software until the cartridge is thawed. Proceed to step 7.6.

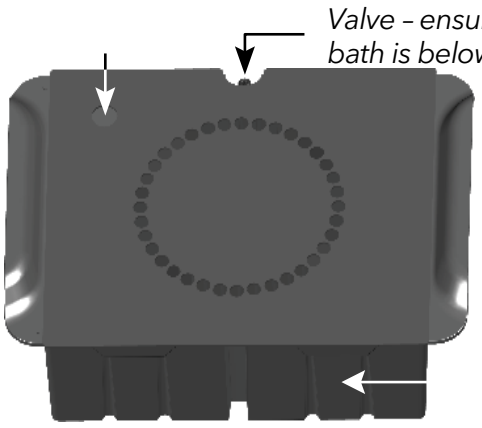
7.6 Prepare for Imaging: Gather Reagents, Thaw Cartridge, and Empty Waste

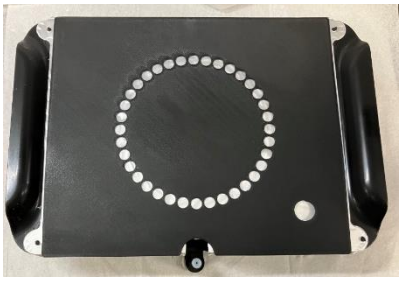
The cartridge, staining reagent, and codebook are specific to the chemistry used in your experiment. Carefully check that all materials align with the chosen chemistry (MERFISH 1.0 or MERFISH 2.0)

Not thawing the correct cartridge will lead to delayed run start and loss of unused cartridge. The software will confirm cartridge compatibility against run configuration.

1. Take Sample Prep Wash Buffer (PN 20300001) and Formamide Wash Buffer (PN 20300002) from the applicable MERSCOPE sample preparation kit (stored at 2-8°C).
2. Prepare a 37°C water bath with ~2 cm (height) of water in the bath (or such that the water

level rises to ~2.5 cm up the outside of the cartridge). Place the applicable MERSCOPE Imaging Cartridge in the 37°C water bath for 60 min. **DO NOT** allow the valve to come into contact with the water.





Not all reagents within the cartridge thaw at the same rate. Incubate in the water bath for the full 60 min.

3. Warm up the Staining Reagent for 10 min in a 37°C water bath. Gently vortex the tube on the lowest setting to ensure the reagents are well mixed, and no precipitate is visible before use. If preparing an experiment using the FCX-L (Large) flow chamber, two vials will be required.
4. Maintain Imaging Buffer Activator (PN 20300022) and RNase inhibitor in a benchtop cooler until use. Spin down using a benchtop centrifuge before use.
5. Return unused reagents to their appropriate storage.
6. Note the MERSCOPE Imaging Cartridge barcode number in case it must be entered manually.
7. Ensure the instrument waste container is empty before starting an experiment.

7.7 Stain Sample

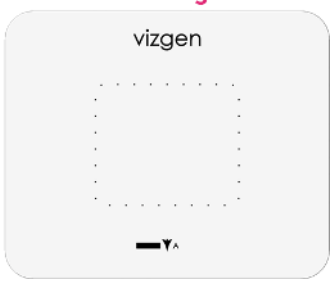
Protect from light *at all times during staining and after the sample is mounted in the MERSCOPE flow chamber, until loaded in the instrument.*

Ensure the staining reagent matches the experimental workflow (MERFISH 1.0 or 2.0).

Formamide Wash Buffer is ***hazardous****. Perform these steps in a fume hood.*

If your MERSCOPE Slide becomes cracked or damaged at any point during the workflow, please halt processing and do not proceed to imaging. A new slide must be prepared for the affected sample.

1. Follow steps in the below table that correspond to the size MERSCOPE Slide you are preparing.

MERSCOPE Standard Slide 	**MERSCOPE Large Slide** 
1. Aspirate the Clearing Solution/Clearing Premix/Formamide Wash Buffer (from sample preparation) ensuring all solution is removed from the petri dish.	1. Aspirate the Clearing Solution/Clearing Premix/Formamide Wash Buffer (from sample preparation) ensuring all solution is removed from the petri dish.
2. Wash **2x** with **5mL** Sample Prep Wash Buffer.	2. Wash **2x** with **10mL** Sample Prep Wash Buffer.
3. Gently vortex the Staining Reagent tube to ensure the reagent is well mixed and no precipitate is visible.	3. Gently vortex the Staining Reagent tube to ensure the reagent is well mixed and no precipitate is visible. Combine the two 3ml tubes provided into a new, separate tube and mix by gently vortexing.
4. Add **3 mL** Staining Reagent. Incubate for **15 min** on a rocker at **ambient** temperature.	4. Add **6 mL** Staining Reagent. Incubate for **15 min** on a rocker at **ambient** temperature.
5. Wash **1x** with **5 mL** Formamide Wash Buffer. Incubate **10 minutes** at **ambient** temperature.	5. Wash **1x** with **10 mL** Formamide Wash Buffer. Incubate **10 minutes** at **ambient** temperature.
6. Wash **1x** with **5 mL** Sample Prep Wash Buffer.	6. Wash **1x** with **10 mL** Sample Prep Wash Buffer.
7. Proceed immediately to the next step.	7. Proceed immediately to the next step.

7.8 Continue Experiment Configuration

NOTE: To run MERFISH 2.0 chemistry, software version 234b or later versions are required. Please make sure MERSCOPE has the compatible software version for running the processed

samples.

1. Click **Next** on the MERSCOPE configuration screen.
    a. *Use **Next** and **Back** to navigate through the configuration screens.*
2. Specify **Imaging Depth**. If unsure of desired depth, 10µm should be selected. For FFPE samples, 10µm should be selected regardless of the thickness of the tissue section collected.
3. The Additional Stains menu is used to define auxiliary bits (Aux [bit number]) based on additional staining that users performed during sample preparation:
    a. *Cell boundary staining*
    b. *Sequential gene encoding with the gene panel*
    c. *Protein staining*

***NOTE**: DAPI and PolyT stains are always imaged automatically.*

4. **IF** the sample was stained with the MERSCOPE Cell Boundary Stain Kit, toggle on **Cell boundary stains** under **Additional Stains**.
    a. *Toggling on **Cell boundary stains** automatically enables Aux 1-3.*
5. **IF** the gene panel contains sequential genes, confirm the corresponding auxiliary bit(s) have been configured accordingly (i.e., **RNA** is selected under **Additional Stains**).
    a. *Navigate to the panel summary page for a constructed gene panel in the MERSCOPE Gene Panel Design Software. Sequential genes are listed along with the assigned auxiliary bits.*
6. **IF** the sample was stained with one or more MERSCOPE Protein Stain Kits, check the corresponding auxiliary bit(s) under **Additional Stains**.

Primary Antibody Host Species	Corresponding Protein Stain	Auxiliary Bit
Mouse	Anti-Mouse Aux 4 Protein Stain	Aux 4
Rabbit	Anti-Rabbit Aux 5 Protein Stain	Aux 5
Goat	Anti-Goat Aux 6 Protein Stain	Aux 6
Rat	Anti-Rat Aux 7 Protein Stain	Aux 7
Human	Anti-Human Aux 8 Protein Stain	Aux 8
Chicken	Anti-Chicken Aux 9 Protein Stain	Aux 9

7. *Users can change the names of additional stains after they are enabled by clicking on the box.*Select the illumination intensity **Protein (Bright)**, **Protein (Medium)**, or **Protein (Dim)** from the menu to the right of each applicable auxiliary bit under **Additional Stains**.
    a. *Users should have established the illumination intensity for each channel during verification.*
    b. *Refer to the MERSCOPE Protein Stain Verification Kit User Guide for more information.*
8. Click **Next**.
9. (**Optional**) Users can set up image processing parameters now and skip segmentation preview.

Note: Segmentation Preview is generated after the experiment has completed imaging and

takes up to 3 hours.

- If image processing parameters are selected during experiment setup, image processing will run automatically once imaging is completed without the need for user intervention following the run (i.e. the Segmentation Preview step will be skipped).
- If image processing parameters are not selected during setup, users will be presented with a Segmentation Preview window after imaging is completed. On this screen, users must select desired parameters to initiate image processing.
- Segmentation options:
  - Cellpose segmentation identifies individual cells by approximating cell boundaries from detected stains and filling cell space from these boundaries. Cellpose segmentation (1, 2, 3) is available if cell boundary stains were used in the measurement.
  - See Vizgen's MERSCOPE Cell Boundary Staining Technical Note for recommendations on selecting the cell boundary stain appropriate for your tissue type.
  - *Cellpose: a generalist algorithm for cellular segmentation, Nat Methods 18, 100-106 (2021).* https://doi.org/10.1038/s41592-020-01018-x

10. Click **Next**.
11. Select Flow chamber:
    - Select either FCX-L (Large) with 47x57 mm rectangular MERSCOPE Slide or FCX-S (Standard) with 40mm round MERSCOPE Slide.

16. Click **Next**.
17. Review the pop-up message and confirm your flow chamber has been accurately selected.

Confirming accuracy of flow chamber selection is critical. Loading a flow chamber that differs from the one selected by the user may result in loss of the experiment and damage to the system.

18. At the end of configuration, **Configure Summary** will appear.
19. If the Configure Summary is satisfactory, click **Next**.

7.9 Load – MERSCOPE Imaging Cartridge Activation and Loading

ENSURE *the format (Standard, Large) of the imaging cartridge selected is the appropriate size for the flow chamber being utilized.*

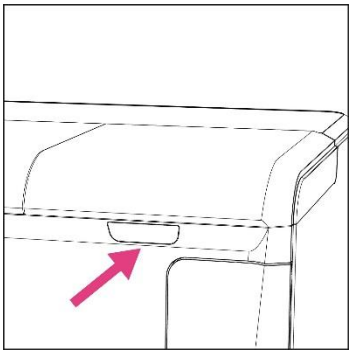
CONFIRM *that the Cartridge matches the chemistry used in experiment – MERFISH 1.0 or MERFISH 2.0 chemistry*

ONLY *activate a MERSCOPE Imaging Cartridge when ready to proceed immediately with an experiment.*

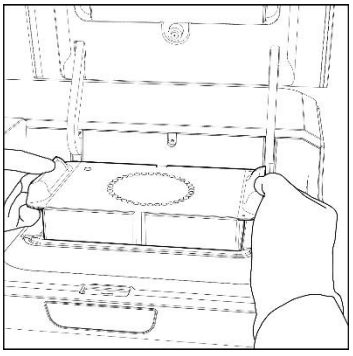
DO NOT *activate a MERSCOPE Imaging Cartridge while it is thawing in the water bath.*

The imaging cartridge contains ***hazardous materials*** *and should be discarded per applicable institutional hazardous waste procedures.*

1. Open the imaging cartridge lid and remove the MERSCOPE Imaging Cartridge from the previous run or MERSCOPE Wash Cartridge from the instrument.



Open the imaging cartridge lid



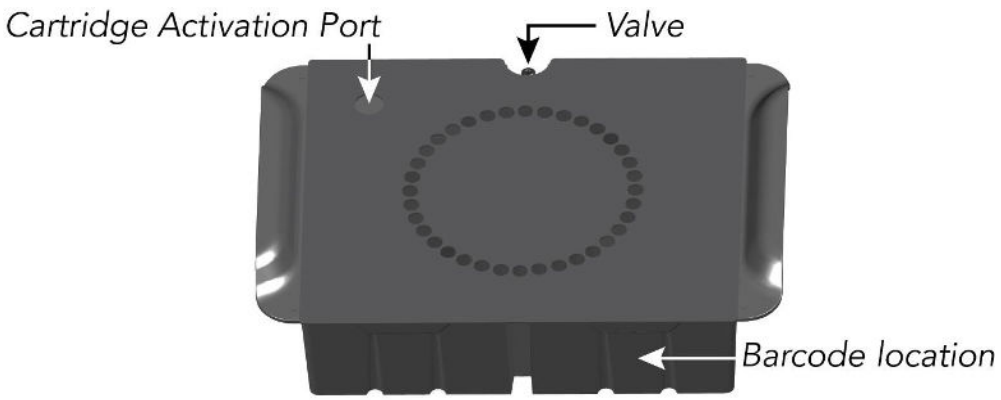
Remove the imaging cartridge from the previous run

2. Remove the new, thawed MERSCOPE Imaging Cartridge from the water bath and dry the outer surfaces, especially the bottom surface. Ensure the barcode is free of any liquid and note the number on the barcode.
3. **Prior to** piercing the Cartridge Activation Port and cartridge activation, slowly invert the thawed MERSCOPE imaging cartridge **10x** to ensure the cartridge reagents are mixed.
4. Clean the MERSCOPE Imaging Cartridge (refer to next page for image) by spraying RNaseZap solution onto a Kimwipe and wiping the valve and foil covering the cartridge activation port. Next spray 70% ethanol onto a Kimwipe and again wipe the valve and foil covering. Users may also clean the connection in the imaging cartridge lid if contamination is a concern.

5. Prepare Imaging Activation Mix by adding 250µL of Imaging Buffer Activator and 100µL of RNase Inhibitor into a new 1mL tube and mix by gentle vortexing.

***NOTE**: Use the same volume of reagents for both Standard and Large Cartridges*.

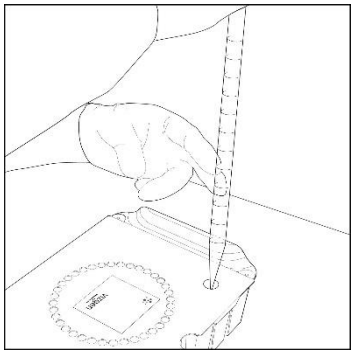
6. **Prior to** inserting the MERSCOPE Imaging Cartridge into the instrument, pierce the foil at the designated Cartridge Activation Port (top left-corner) with a clean pipette tip. **Ensure** the foil in the port is completely open.



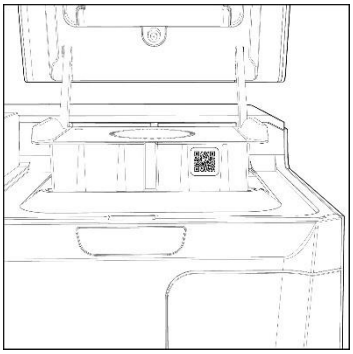
7. With a 1-mL pipette set to 1 mL, carefully add all the Imaging Activation Mix via the Cartridge Activation Port by inserting the pipette tip below the level of the liquid in the MERSCOPE Imaging Cartridge before dispensing. **Without changing** the pipette tip, lower and raise the pipette plunger **10x** at moderate speed to thoroughly mix the solution in the imaging cartridge, but without introducing air bubbles.
8. With a 25-mL serological pipette, carefully layer **15 mL** mineral oil over the liquid in the MERSCOPE Imaging Cartridge via the Cartridge Activation Port.

***DO NOT** invert the imaging cartridge after the Cartridge Activation Port has been punctured.*

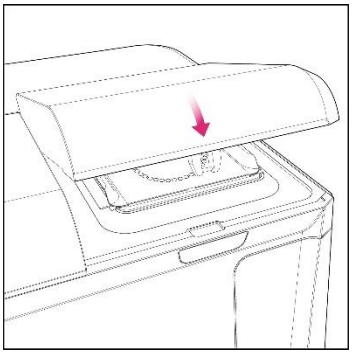
9. Again, ensure the barcode on the MERSCOPE Imaging Cartridge is free of any liquid, and the outside/underside of the cartridge is also dry. Insert the activated imaging cartridge into the instrument with valve toward the back and the barcode toward the front (see picture below).



Activate the imaging cartridge lid and layer mineral oil in the imaging cartridge



Ensure the activated imaging cartridge barcode is clean and facing the front of the instrument. Insert the activated imaging cartridge into the instrument



Close the imaging cartridge lid

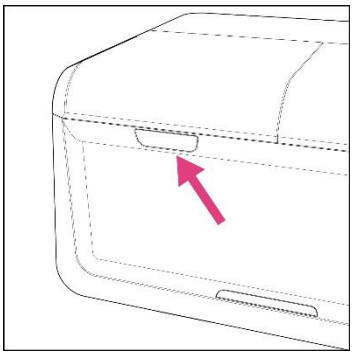
10. Close the imaging cartridge lid and click **Scan Barcode**.
    a. *The instrument scans the MERSCOPE Imaging Cartridge barcode for compatibility with the selected MERSCOPE Codebook. If the instrument cannot read the MERSCOPE Imaging Cartridge barcode, the barcode number may be entered manually.*
11. If the barcode validation is successful, click **Prime Fluidics**.
    a. *The instrument will proceed to prime the fluidics (this takes 2-3 min).*

7.10 Load – MERSCOPE Ultra Flow Chamber

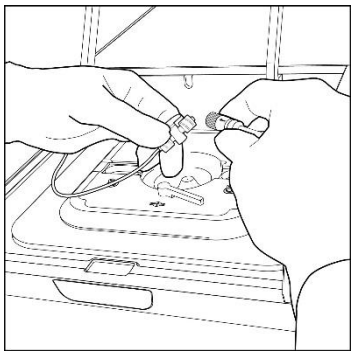
In general, the MERSCOPE Flow Chamber should be lifted out of, and placed into, the stage adapter by gently holding the fluidic lines to either side of the flow chamber and lifting/placing vertically (i.e., do not tilt the aqueduct).

*Click **Next** to advance through screens as operations are performed.*

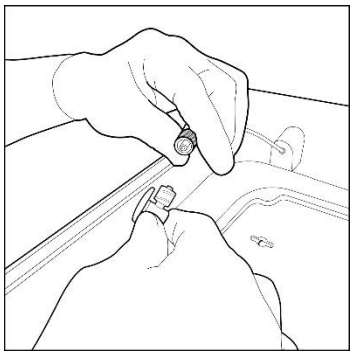
1. Open the flow chamber lid.
2. Disconnect the Fluidic Line Connector from the input line and the output line.



Open the flow chamber lid



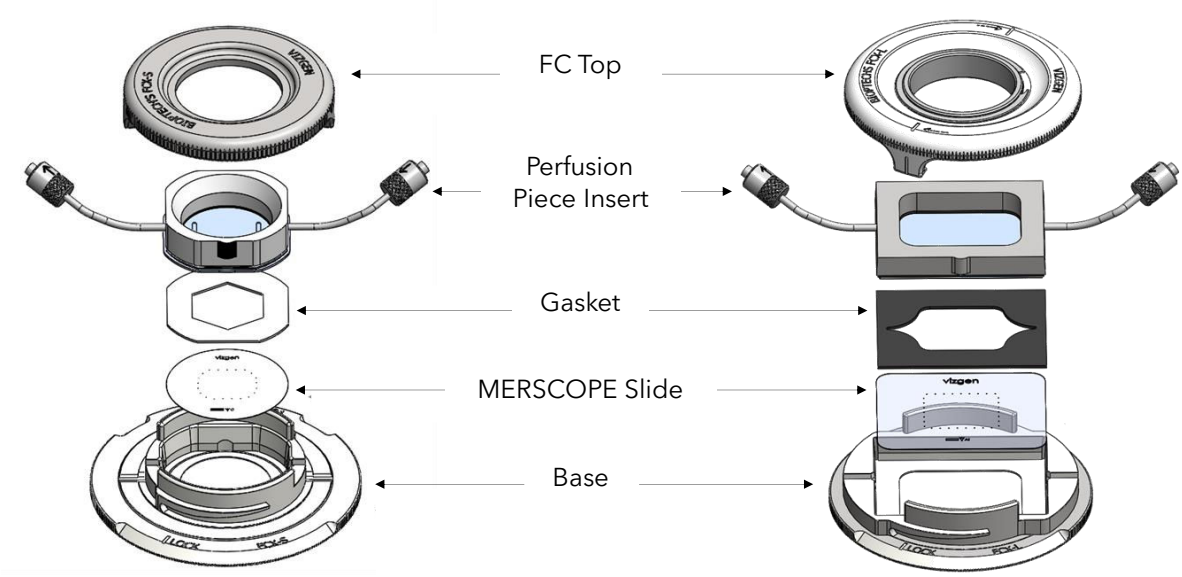
Disconnect the Fluidic Line Connector from the input line



Disconnect the connector from the output line

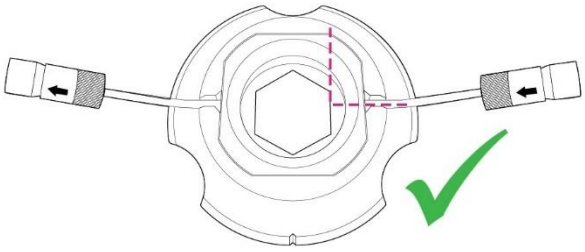
3. Rotate the MERSCOPE Flow Chamber Top counterclockwise to disassemble the flow chamber (refer to next pages for images) and clean the gasket, base, and aqueduct by spraying with RNaseZap solution and wiping with a Kimwipe, followed by spraying with 70% ethanol and wiping with a Kimwipe.
4. Hold the MERSCOPE Flow Chamber base close to the sample petri dish. Gently pick up the MERSCOPE Slide with tweezers and place into the base (sample gel facing up).
5. Assemble the MERSCOPE flow chamber by placing the gasket on top of the MERSCOPE Slide (refer to next page for image of correct gasket orientation).
6. Assemble the aqueduct and top. Ensure the notch in the base and the flow direction arrows marked on the Aqueduct connectors are oriented correctly (refer to next pages for images). Twist the top clockwise until the **Lock** alignment markings on the base and top are aligned.
7. Once assembled, spray the bottom of the MERSCOPE Slide with 100% ethanol and wipe clean with lens paper. Repeat **2x** more (3x total) to ensure the bottom imaging surface of the MERSCOPE Slide is clean.

MERSCOPE Flow Chamber Components and Gasket Orientation



FCX-S **FCX-L**

FCX-S



The gasket should be oriented with a long side of the hexagon positioned perpendicular to the fluid input line

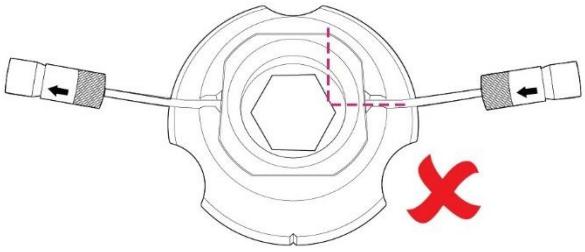
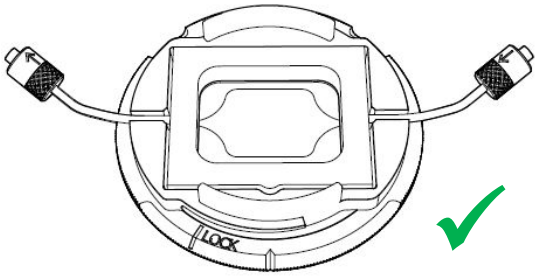


Illustration of an INCORRECT orientation of the gasket

FCX-L



The gasket should be oriented with the short side of the gasket positioned perpendicular to the fluid input line

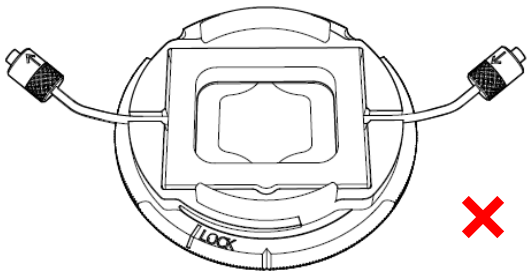
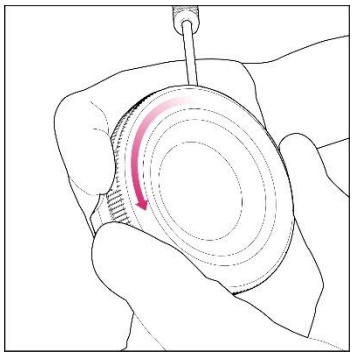
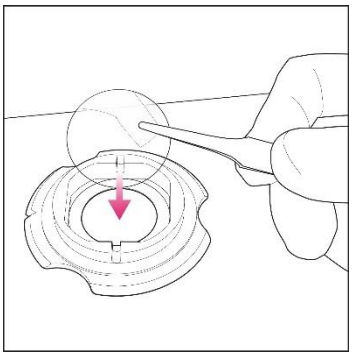


Illustration of an INCORRECT orientation of the gasket

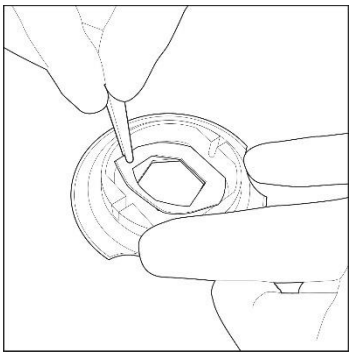
MERSCOPE Flow Chamber FCX-S (Standard) Assembly and Cleaning



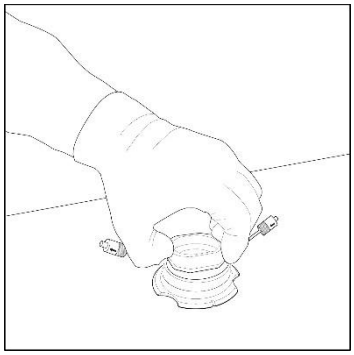
Rotate the Top counterclockwise to disassemble the flow chamber. Clean the gasket, base, and aqueduct



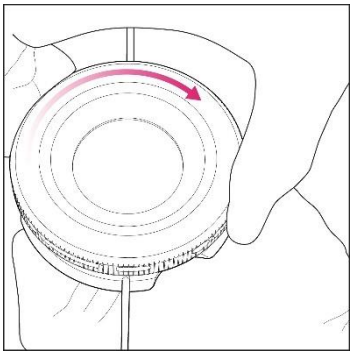
Place the new MERSCOPE Slide in the base with sample gel facing up



Place the gasket on top of the MERSCOPE slide. Ensure orientation is correct.

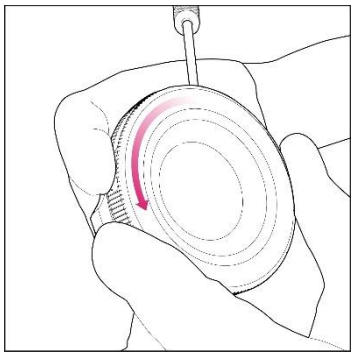


Insert the aqueduct. Ensure the notch in the base and flow direction arrows are oriented correctly.

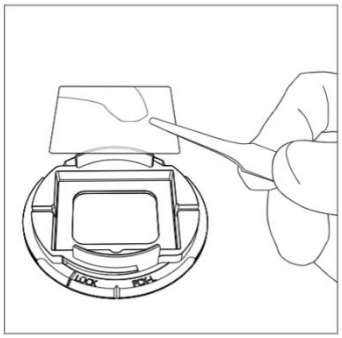


Align and twist the top clockwise until the **Lock** markings on the top and base are aligned.

MERSCOPE Flow Chamber FCX-L (Large) Assembly and Cleaning

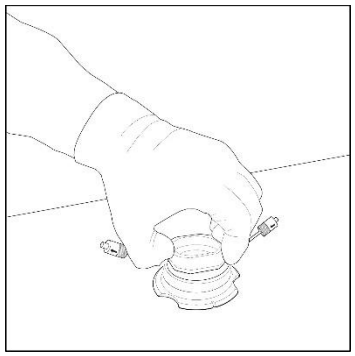


Rotate the Top counterclockwise to disassemble the flow chamber. Clean the gasket, base, and aqueduct

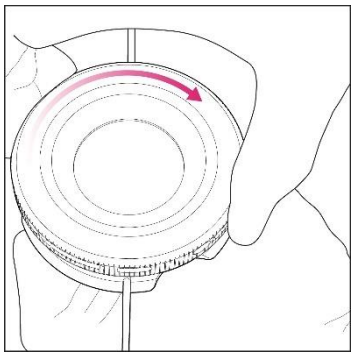


Place the new MERSCOPE Slide in the base with sample gel facing up

Place the gasket on top of the MERSCOPE slide. Ensure orientation is correct.

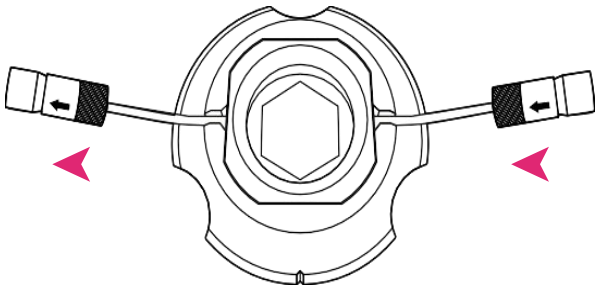


Insert the aqueduct. Ensure the notch in the base and flow direction arrows are oriented correctly.



Align and twist the top clockwise until the **Lock** markings on the top and base are aligned.

The MERSCOPE Instrument pulls fluid from right to left (pink arrows indicate the direction of flow).



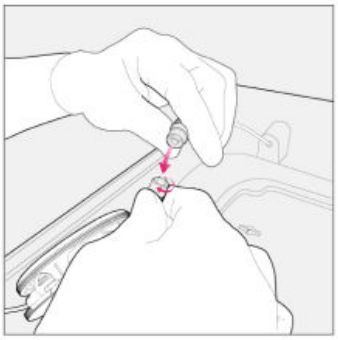
Label of "LOCK" and "FCX-S/L" front of instrument

It is critical to insert the MERSCOPE Flow Chamber into the instrument in the correct orientation.

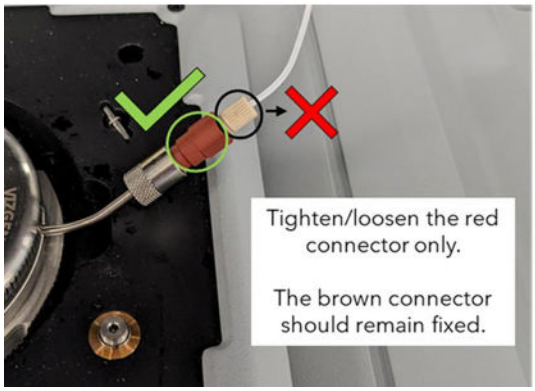
The aqueduct has flow direction arrows marked on the connectors (illustrated without the Top in place).

The flow chamber should be connected to and inserted into the instrument with the notch Label of "LOCK" and "FCX-L/S" in the Base toward the front of the instrument.

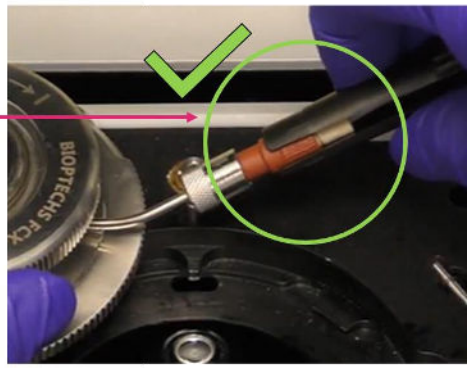
9. Connect the assembled MERSCOPE flow chamber to the instrument fluidic lines. First, connect the output line (to the left). Then connect the input line (to the right).



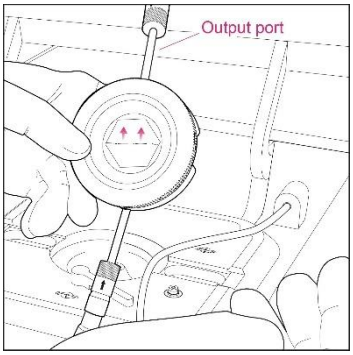
10. When connecting the instrument fluidic lines, it is important to note that only the red connector should be rotated to secure the fitting to the flow chamber. The brown adapter adjacent to the red connector should not be rotated. A torque adapter is provided in the instrument accessories kit that should be used when tightening the red connector to ensure it is not over tightened. The inlet/outlet tubing should be slid into the slit and the threaded end of the torque adapter secured over the back of the red connector. Turn the torque adapter clockwise to tighten until you hear an audible click. Remove the torque adapter by sliding it away from the fitting. See images below.



Tighten red connector using the provided torque adapter



11. Click **Wet Flow Chamber** to initiate wetting the MERSCOPE flow chamber. Orient the flow chamber vertically with the output lines upwards as flow starts to pull the air bubbles through the flow chamber and fluidic lines (refer to image below). If air bubbles are seen during wetting, rotate and tap the side of the flow chamber to force the bubbles into the outlet port.



Orient the flow chamber vertically with the output lines upwards while filling

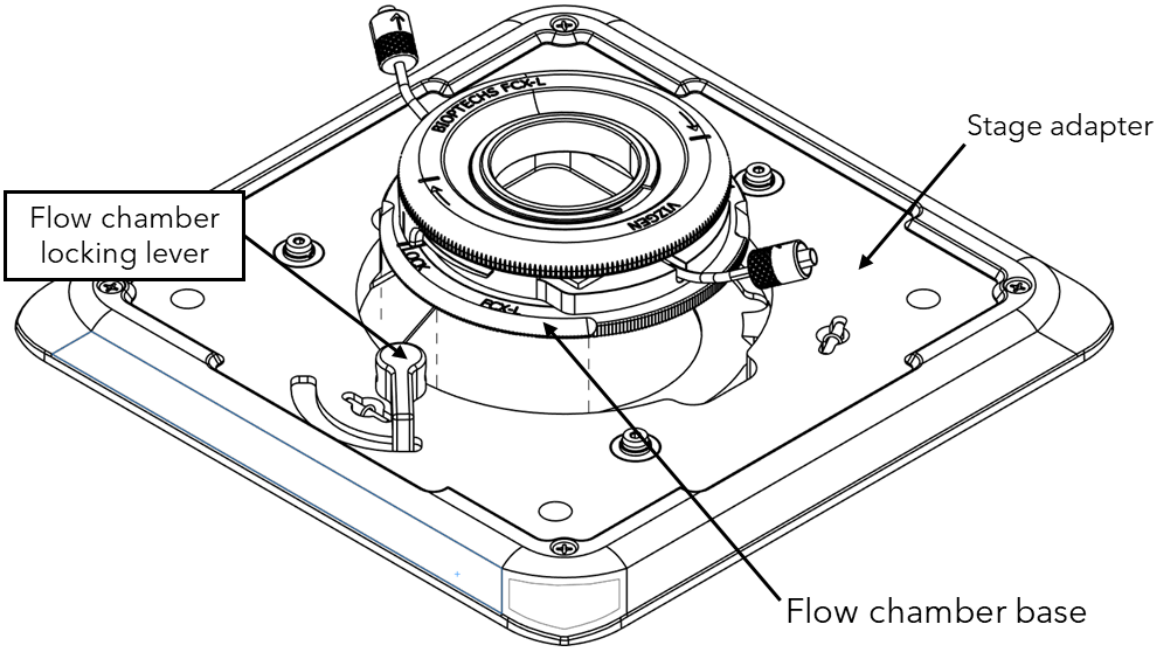
12. If air bubbles are visible in the MERSCOPE flow chamber or input line, click **Pull more liquid**. If/when no air bubbles remain, click **Next**.

*It is only possible to **Pull more liquid** twice. If air bubbles are still visible after pulling more liquid once, ensure the fluid connections are correctly assembled and tightly closed before pulling liquid for a second time.*

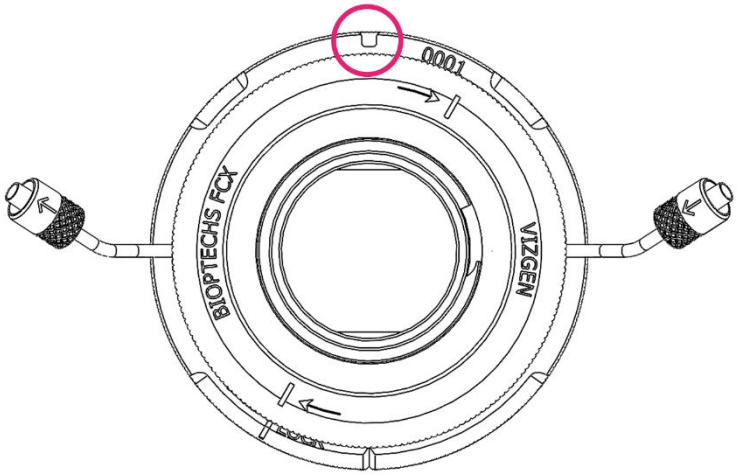
If air bubbles remain after pulling liquid for a second time, contact Vizgen Support (support@vizgen.com).

13. Insert the MERSCOPE flow chamber into the instrument.

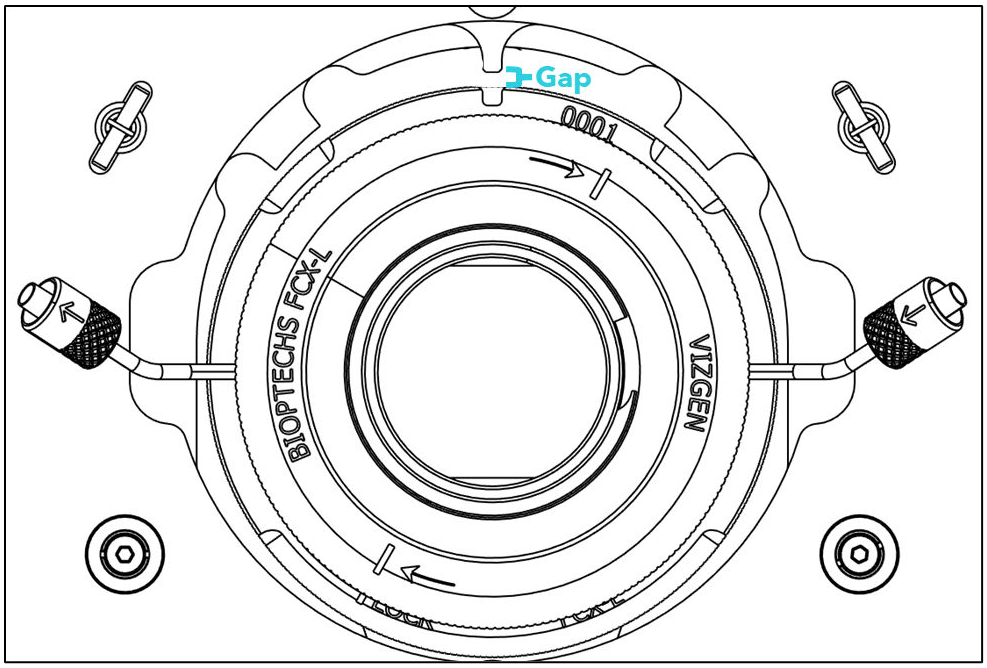
Flow Chamber Interface & Components



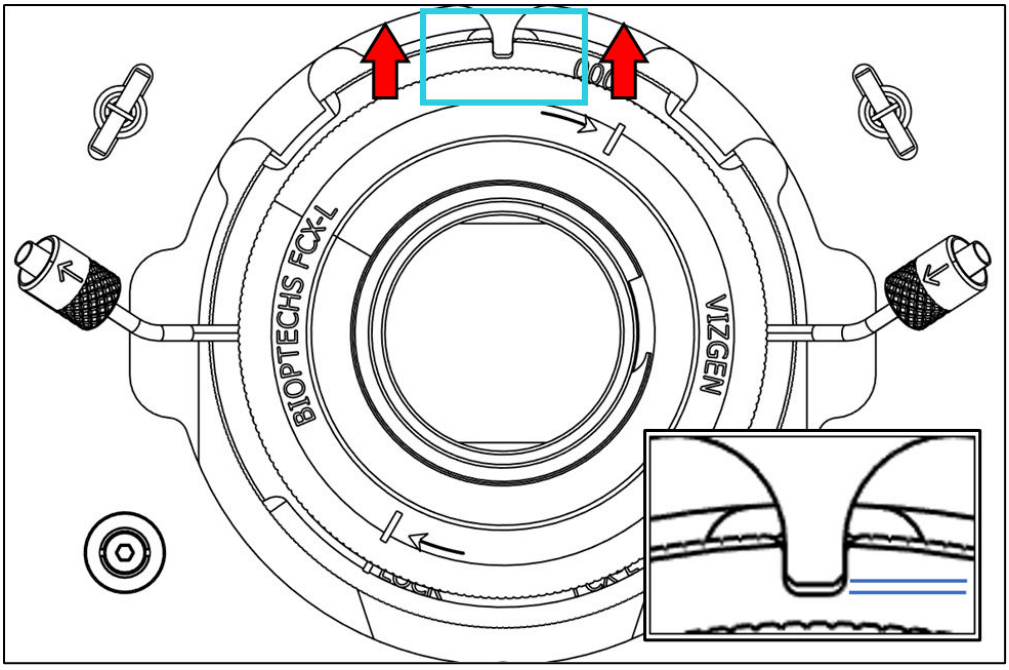
14. Start by locating the notch on the top plate of the flow chamber base (circled below) and orient the flow chamber so the notch is at the 12 o'clock position. Also, confirm the flow chamber locking lever on the stage adapter is in the open (counterclockwise) position.



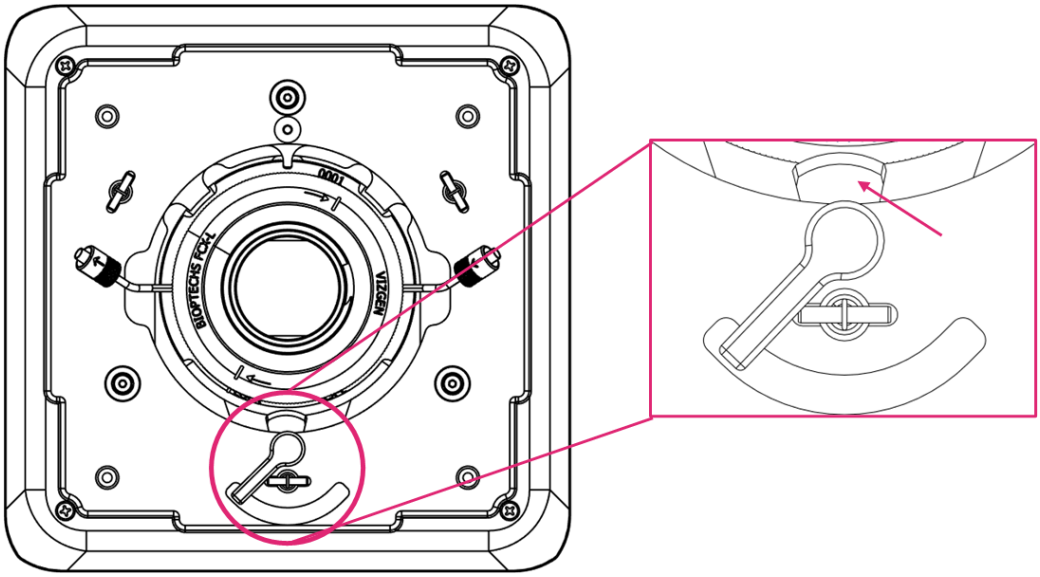
15. Lower the flow chamber into the stage adapter by first inserting the back of the flow chamber under the overhangs at 10 and 2 o'clock then rest the front of the flow chamber flat on the stage adapter. Looking down at the flow chamber, it should be approximately positioned as shown below.



16. Once the flow chamber is lying flat on the stage adapter, gently push the flow chamber toward the 12 o'clock position so the notch is engaged with the tab on the stage adapter. Note: By design, the tab will not sit flush against in the notch. Below image and the inset shows proper positioning and approximate gap size, respectively. If there is no gap, or if the gap is excessive, the flow chamber has not been installed correctly.



17. Lock the flow chamber into place by rotating the locking lever clockwise. The lever should be tightened by hand until firm resistance is felt. The lever may not travel to the end of the groove. Overtightening may cause damage to the locking mechanism. When tightened appropriately, the flow chamber will be secure, and the brass locking mechanism will be visible against the 6 o'clock position of the flow chamber base (circled below).
    a. *If the locking mechanism will not engage, it is possible that the MERSCOPE flow chamber has not been inserted correctly under the overhangs in the stage adapter. Remove the flow chamber from the stage adapter and try again.*



18. Feed any excess inlet and outlet tubing back into the instrument to avoid the tubing from getting caught when closing lid.
19. Close the flow chamber lid and click **Acquire Mosaic** to acquire a low-resolution mosaic.

NOTE: acquisition of low magnification mosaic takes ~30 minutes.

7.11 Select Regions of Interest

The instrument will acquire a low-resolution mosaic using a low-magnification

1. Select the regions of interest to be included in the experiment using the touchscreen or mouse. Draw a boundary on the mosaic to define the region of interest for MERFISH imaging.
    a. *Once a boundary is drawn, it is saved, and a summary appears on the right-hand side of the screen.*
2. Drawing another boundary automatically creates a new region.
3. Select an existing region by clicking on it on the right-hand side of the screen. When a region is selected, hold and drag a boundary dot to change its location (to redefine the boundary). Click **Done** to exit out of a selected region.

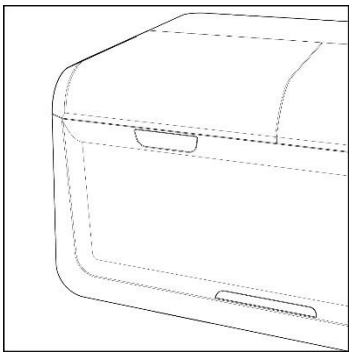
    If regions are drawn outside the imaging area, the software will provide a Constrain Region button next to the corresponding region. Use this button to automatically adjust the region

within the acceptable area. Alternatively, users may edit the region manually to resolve the issue.

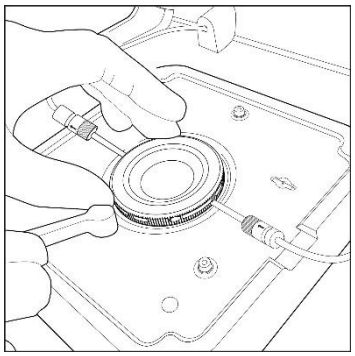
4. The regions can be renamed by clicking the pencil icon next to the region name.
5. Up to 10 regions can be selected with a **total area of up to 125 mm²** (1.25 cm^2) for FCX-S (Standard) and **up to 300 mm²** (3.0 cm^2) for FCX-L (Large).
6. If needed, use the **Visible Intensity Range** slider to adjust the contrast of the image.
7. When selections are complete, click **Next**.

7.12 Switch to the High-Magnification Objective

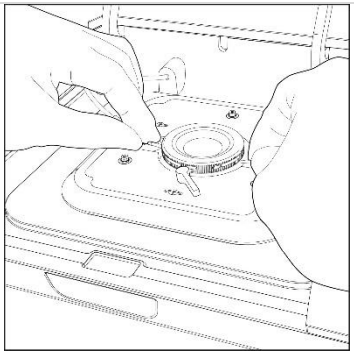
1. Open the flow chamber lid and unlock and remove the MERSCOPE flow chamber from the stage adapter but **DO NOT DETACH** the fluidic lines. Click **Next** to advance through screens as operations are performed.



Open the flow chamber

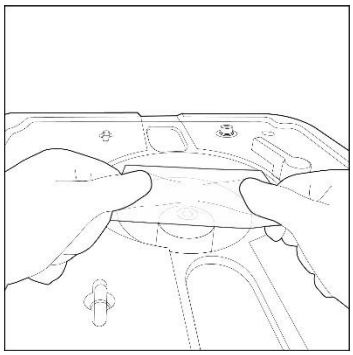


Unlock the flow

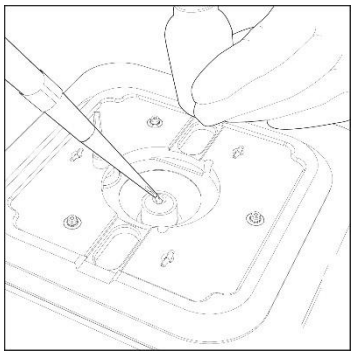


Remove the flow chamber from the stage adapter but **DO NOT DETACH** the fluidic lines

2. Clean the immersion oil from the high-magnification objective with lens tissue.
3. Pipette **75 µL** (4 drops) fresh immersion oil onto the high-magnification objective. To ensure there are no air bubbles in the immersion oil, pipette the viscous liquid slowly and hold the pipette tip in the immersion oil for at least 20 sec. If air bubbles are present, clean off the immersion oil using lens tissue and repeat the application with fresh immersion oil.



Clean the high-magnification objective with lens tissue



Pipette **75 µL** (4 drops) immersion oil onto magnification objective. Check for bubbles

4. Re-insert the MERSCOPE flow chamber into the instrument. Lower the flow chamber into the stage adapter by first inserting the back of the flow chamber under the overhangs at 10 and 2 o'clock then rest the front of the flow chamber flat on the stage adapter. Looking down at the flow chamber, it should be approximately positioned as shown below. Then, push the flow chamber all the way back to bottom out within the stage adapter.

   Then, push the flow chamber all the way back to bottom out within the stage adapter.
5. Lock the MERSCOPE flow chamber into place.
6. Feed any excess inlet and outlet tubing back into the instrument to avoid the tubing from getting caught when closing lid.
7. Close the flow chamber lid and click **Acquire Focus**.

7.13 Experiment – Data Acquisition

The instrument will attempt to find the focal plane with the high-magnification objective.

1. If the focusing is successful, click **Next** to advance to an **Experiment Summary**.

If the instrument is unable to find the focal plane or detects an air bubble in the immersion oil, the user interface will instruct users to try again with immersion oil application.

2. If the **Experiment Summary** is satisfactory, click **Start Measurement** to initiate the fully-automated experiment.
   - *The instrument calculates the estimated time to image the selected areas. The total instrument time depends on the selected area and size of the gene panel.*
   - *The instrument will automatically run the sample. A progress bar reports progress within each imaging round.*
   - *The instrument will indicate **Done!** when all measurements are complete.*

***DO NOT** power cycle the MERSCOPE Ultra Analysis Computer while image analysis is in process.*

7.14 Segmentation Parameters and Image Processing (optional)

1. If image processing parameters are selected during experiment setup, the run will proceed to clean and image processing automatically.

   Otherwise, **Segmentation Parameters** can be selected after imaging has been completed. The Segmentation Parameters screen allows users to select different fields of view to evaluate segmentation parameters in each field of view. Segmentation options:
   - Cellpose segmentation (*Cellpose: a generalist algorithm for cellular segmentation, Nat Methods 18, 100-106 (2021).* https://doi.org/10.1038/s41592-020-01018-x)
2. Select one of the two options for segmentation including the boundary stain.
3. Segmentation results will be displayed on the image below. On the left side, the image channel displayed may be selected and the Visible Intensity Range slider may be adjusted to facilitate evaluation of the segmentation results.

- *After segmentation parameters are defined, an Image Processing Parameters screen will appear.*

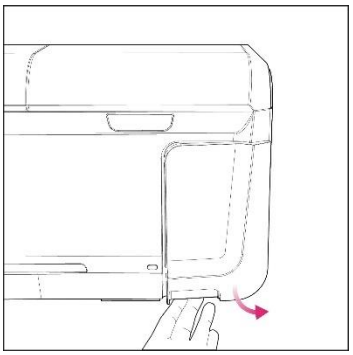
4. If the **Image Processing Parameters** are satisfactory, click **Start Image Processing** to initiate image processing analysis.
5. Once image processing analysis is complete, the results will be available at Z:\merfish_ output and should be copied off the instrument either through the 10 Gb ethernet port or through a portable hard drive plugged into the USB port on the front of the instrument to ensure available storage for new experiments.
6. Click **Clean Instrument** to proceed to system cleaning. The Clean process and a new experiment can begin on the instrument while image processing analysis of previous experiments is running in the background.

7.15 Clean

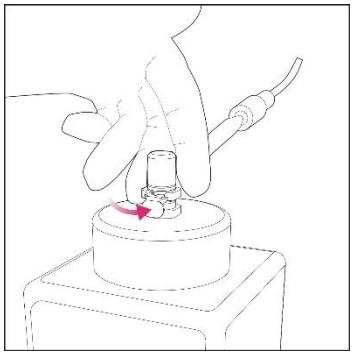
***IF** the instrument will not be used to run a MERFISH experiment for ≥2 weeks,or if verification is the only experiment planned for ≥2 weeks, prepare the instrument per the MERSCOPE Ultra Instrument Idle Procedure **IN ADDITION TO (AFTER)** the Clean procedure.*

1. The instrument executes automatic fluidic line washes. The user interface also guides users through (1) emptying the waste container, (2) removing the MERSCOPE Flow Chamber and cleaning the immersion oil off the high-magnification objective with lens tissue, and (3) installing a Fluidic Line Connector (PN 60900123) after an experiment is complete. Click **Next** to advance through screens as operations are performed. Refer to the next page for images.
2. After the MERSCOPE Flow Chamber is removed from the instrument, rotate the flow chamber Top counterclockwise to disassemble the flow chamber. Lift the Aqueduct vertically (i.e., do not tilt during removal). Discard the MERSCOPE Slide per applicable institutional hazardous waste procedures.
3. Prepare the flow chamber for the next run (Load – MERSCOPE Flow Chamber) or for storage (MERSCOPE Ultra Instrument Idle Procedure). If not proceeding immediately to next run, clean the gasket, base, and aqueduct by spraying with 70% ethanol and wiping with a Kimwipe.
4. Discard the waste container contents per applicable institutional hazardous waste procedures.
   - *The waste container must weigh less than 250 g for the instrument to proceed with the next run*
   - *Reconnect the waste bottle – instrument will not initialize if waste bottle is not properly connected*
5. At the end of cleaning, click **Done** to return to the home page. To maintain optimal performance of system fluidics, it is recommended users also perform an **Instrument Wash** after the Clean procedure is complete. Follow the steps in INSTRUMENT WASH (MAINTENANCE) section to perform this step.

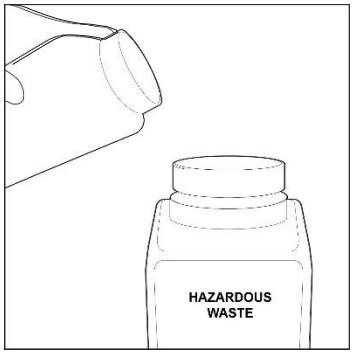
Clean Instrument Step 1 of 3 – Empty waste



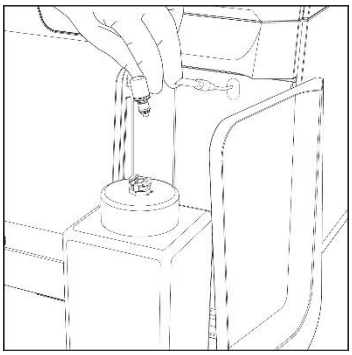
Open the waste container door



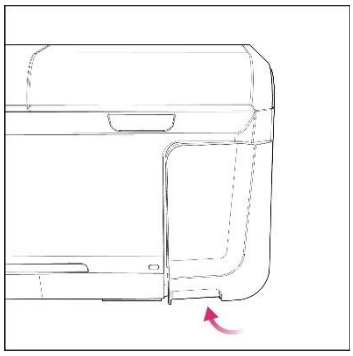
Disconnect the waste container tube



Empty the waste container

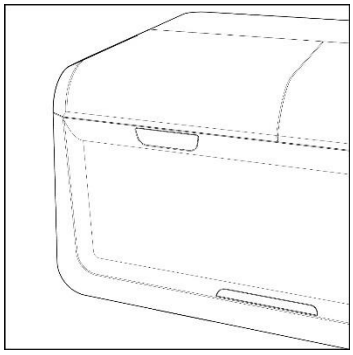


Reconnect the waste container door

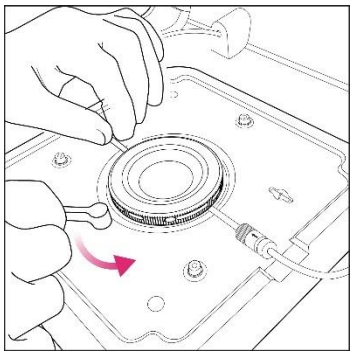


Close the waste container

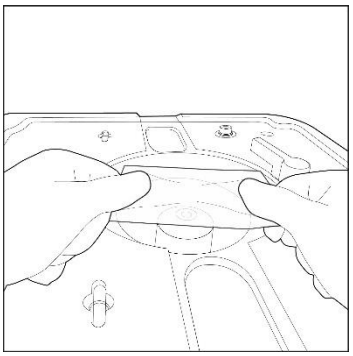
sInstrument Step 2 of 3 – Remove oil from the high-magnification objective



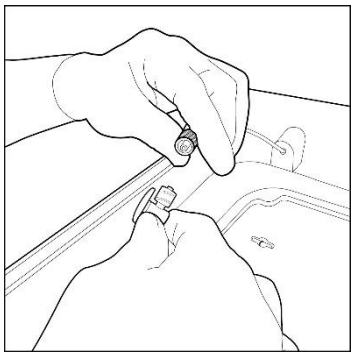
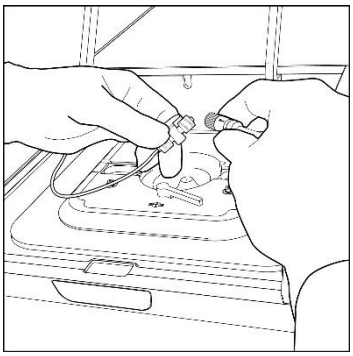
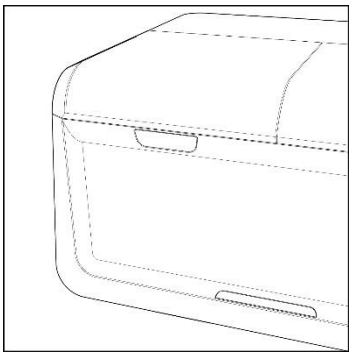
Open the flow chamber



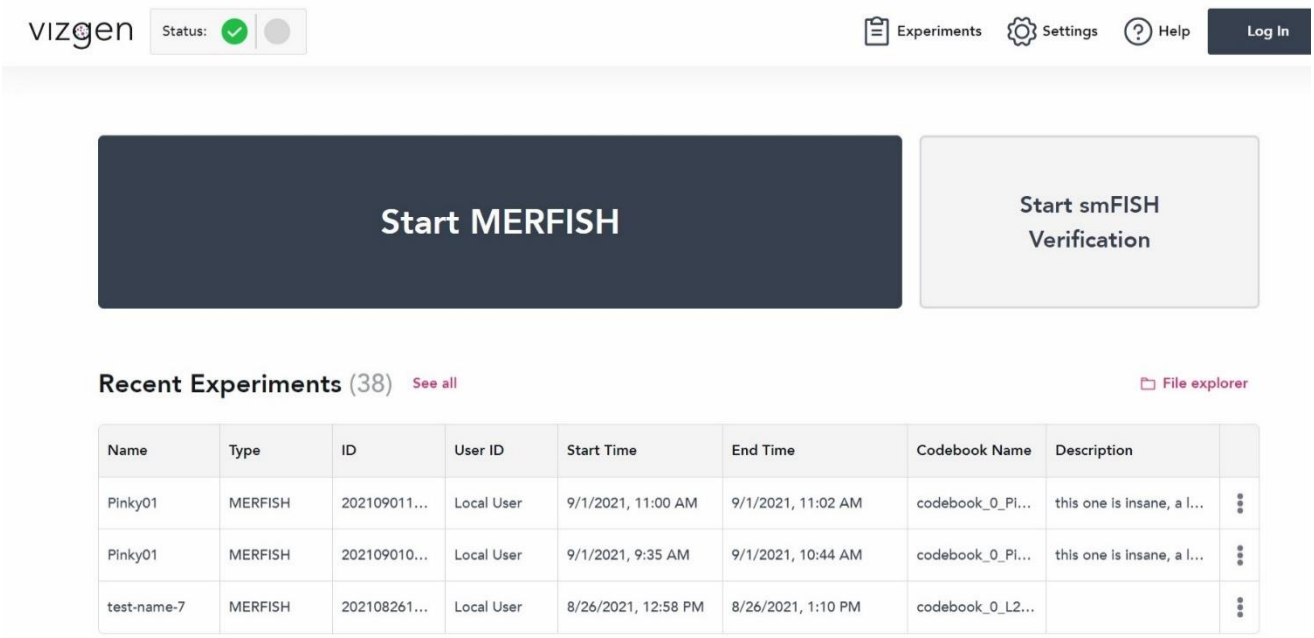
Unlock the flow chamber. Remove the flow chamber from the stage adapter and disassemble



Clean the high-magnification objective with lens tissue

Clean Instrument Step 3 of 3 – Connect the fluidic lines		
		
Connect a Fluidic Line Connector to the output line	Connect the connector to the input line to complete the circuit Ensure the tap of the connector is in the open orientation	Close the flow chamber lid

8 NAVIGATING THE HOME SCREEN



8.1 Home Page Status Indicators

1. **Compression**. Users can check the status of raw image compression for current and queued experiments. **Analyzer**. Users can check the connection with their processing controller and check the status of image processing for current and queued experiments

The following table provides an overview of the different states displayed:

Module	Icon	Meaning	Examples
Compression		System OK, performing as expected	N/A
		Warning	Compression drive is full large backlog
		Error event	Disconnected, Software error
Analyzer		System OK, performing as expected	N/A
		Error event	Hardware or software issue/failure

8.2 Home Screen Top Navigation Bar

1. **Experiments**. Users can browse prior experiments.
2. **Maintenance**. Wash the instrument fluidic lines.
3. **Settings**. Users can confirm available disk space and software version.
4. **Help**. Users can view the system user guide.
5. **Log In**. Users must log into their account to have access to prior experiments and MERSCOPE Codebooks via the Vizgen Cloud.

8.3 Initiation of MERFISH Experiment Workflow

Workflow walks the user through configuring and loading a sample for MERFISH imaging.

8.4 Initiate Sample Verification Experiment Workflow

Workflow walks the user through configuring and loading a sample for RNA or Protein verification.

9 INSTRUMENT WASH (MAINTENANCE)

It is recommended that users perform an instrument wash before and after every MERFISH experiment to maintain optimal performance of system fluidics.

Users may wash the instrument fluidic lines by implementing the wash program under **Maintenance** on the home page top navigation bar. A power cycle is required to perform the instrument wash.

In addition to maintenance, the wash program should also be performed as part of INSTRUMENT SHUTDOWN (IDLE) AND RESTART.

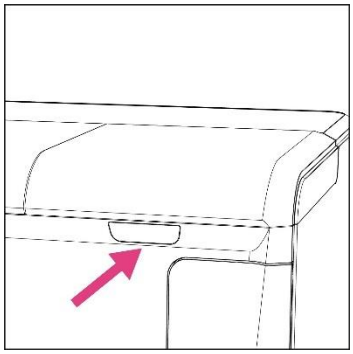
1. Power cycle the system by:
    a. Shutting down the Instrument Computer and turn the toggle switch on the back of the Control Box to the "Off" position.
    b. Waiting 60 seconds

c. Switch the toggle switch on the back of the Control Box into the "On" position.

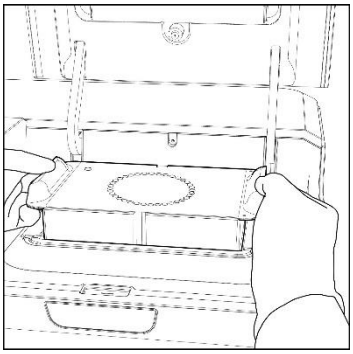
2. Double click the MERSCOPE application icon to open the instrument user interface.

NOTE: The data compression service takes a few minutes to initialize at startup and will show an error state for the Compression service on the Home Page Status Bar until initialization is complete.

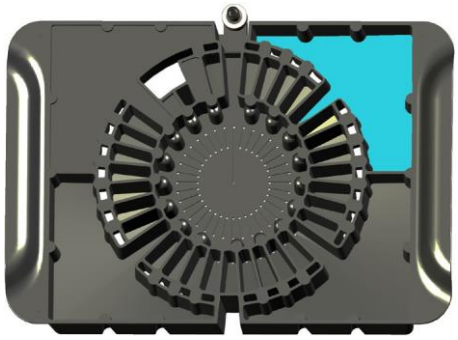
3. Click **Start instrument wash** and click **Next** to advance through screens as operations are performed.
4. Open the imaging cartridge lid and remove the MERSCOPE Imaging Cartridge from the previous run from the instrument. The imaging cartridge contains hazardous materials and should be discarded per applicable institutional hazardous waste procedures.
   a. *The user interface guides users through removal of the MERSCOPE Flow Chamber, cleaning the high-magnification objective, and installation of Fluidic Line Connector in case it was not performed as part of the last Clean procedure.*
5. Open the flow chamber lid and ensure the tap of the Fluidic Line Connector is in the open orientation. Close the flow chamber lid.
6. Rinse the back right well of a MERSCOPE Wash Cartridge (PN 10700102) with nuclease-free water, where the valve indicates the back of the wash cartridge (refer to next page for image). Then, fill the back right well of the wash cartridge with **30 mL** nuclease-free water.
7. Insert the filled MERSCOPE Wash Cartridge into the instrument and close the imaging cartridge lid.



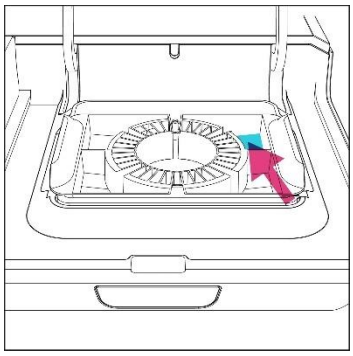
Open the imaging cartridge lid



Remove the imaging cartridge from the previous run

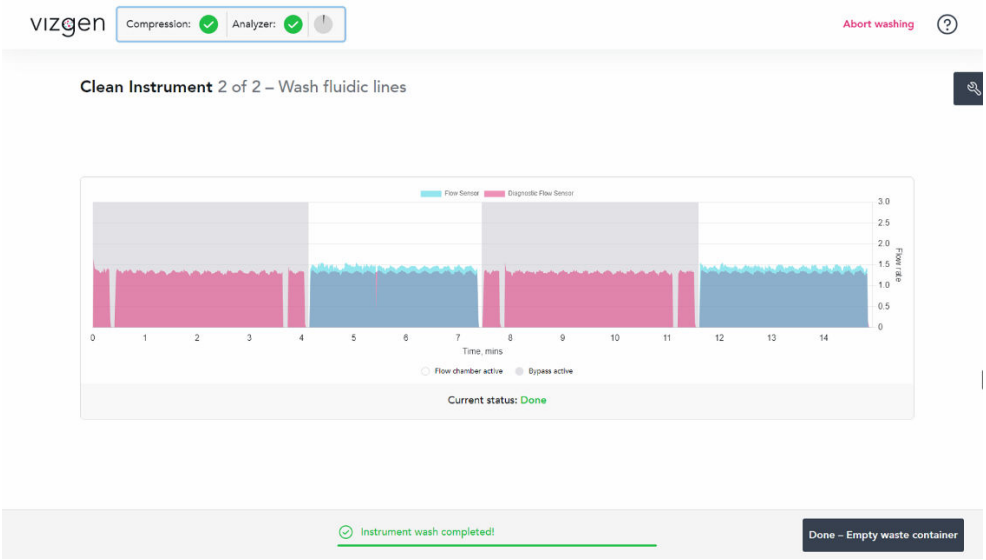


Rinse the back right well of a MERSCOPE Wash Cartridge with nuclease-free water. Then, fill the back right well with 30 mL nuclease-free water, where the valve indicates the back of the cartridge

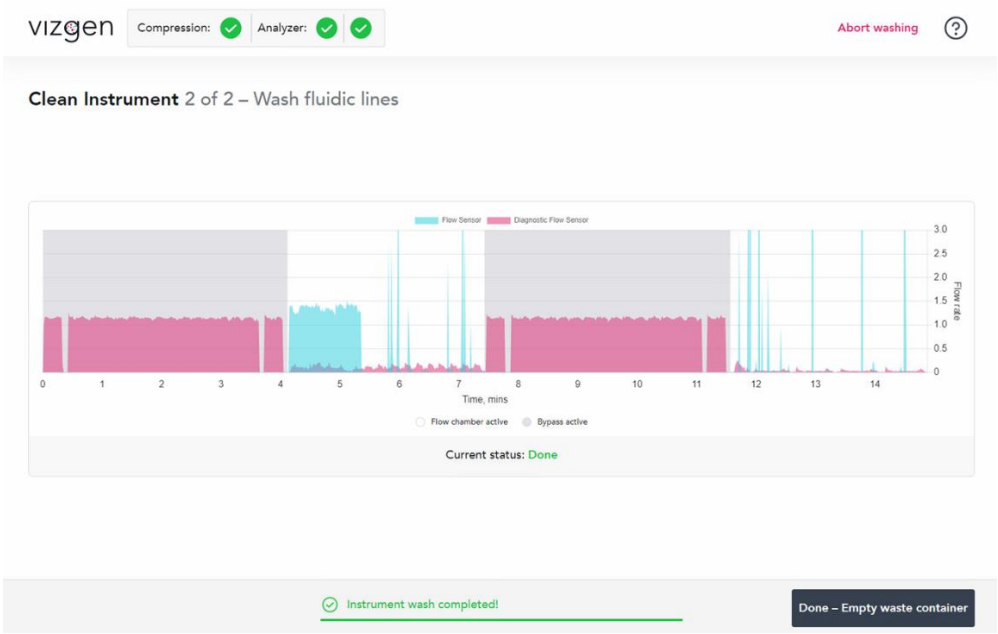


Insert the wash cartridge with 30 mL nuclease-free water into the instrument and close the imaging cartridge lid

8. Click **Start Fluidics Line Wash** to initialize the instrument wash.
   – *A progress bar reports progress.*
9. Determine if the flow rate is acceptable by observing the flow rate during minutes 6 - 7 and 12 - 15.
   a. The flow rate is acceptable if there are no periods of ≥1 min in which the flow rate is out of the range of 1.0 to 2.0. Proceed with an experiment per normal operations.
   b. The flow rate is **NOT** acceptable if the flow rate is out of the range of 1.0 to 2.0 for ≥1 min.
   c. If the flow rate is out of the range of 1.0 to 2.0 for ≥1 min, first inspect the fluidic connector tightening, then repeat the maintenance wash with **10 mL** 70% ethanol. The ethanol wash will remove any chemical residual on the flow sensor.
   d. Repeat the maintenance wash with 30 mL water. Only inspect the flow rate in the latter water wash cycle.
   e. Contact Vizgen Support (support@vizgen.com) if flow rates are still outside of the expected range.



Acceptable flow rate example. During minutes 4-7 and 12-15, there are no periods of ≥1 min where the flow rate is out of the range of 1.0 to 2.0.



Unacceptable flow rate example. During minutes 4-7 and 12-15, there are multiple periods of ≥1 min where the flow rate is out of the range of 1.0 to 2.0.

10. Click **Done – Empty Waste Container** when the instrument wash is complete.
11. Empty the waste container per the instructions in the Clean section.
12. Click **Done – Go to Home Page** to return to the home page. When all maintenance washes are complete:
13. Leave the Fluidic Line Connector in place until the next run.
14. Store the wash cartridge upside down on a Kimwipe or other lint-free wipe inside a drawer.
15. Ensure both the flow chamber lid and imaging cartridge lid are closed.

10 INSTRUMENT INSTALL AND OPERATION

10.1 Initial Install and Startup

Initial MERSCOPE Ultra Instrument installation and startup is performed by an authorized Vizgen representative. Refer to the *MERSCOPE Ultra Instrument Site Preparation Guide* for more information, available online at https://vizgen.com/.

10.2 Normal Day-to-Day Operations

After an experiment is complete, the user interface returns to the home page and users can proceed with the next experiment, even while image processing analysis is running on the previous experiment. It is recommended that an Instrument Wash is performed following each MERFISH experiment. Prior to running a subsequent experiment, the system should be power cycled and an Instrument Wash performed.

11 INSTRUMENT SHUTDOWN (IDLE) AND RESTART

11.1 MERSCOPE Ultra Instrument Idle Procedure

IF *the instrument will not be used to run a MERFISH experiment for ≥2 weeks, or if only verification runs are planned for ≥2 weeks, prepare the instrument as outlined below.*

1. Perform the cleaning process per normal operations after the final experiment.
2. Prepare the MERSCOPE Flow Chamber:
    a. After the MERSCOPE Flow Chamber is removed from the Instrument, rotate the MERSCOPE Flow Chamber Top counterclockwise to disassemble the flow chamber. Lift the Aqueduct vertically (i.e., do not tilt during removal). Discard the MERSCOPE Slide per applicable institutional hazardous waste procedures.
    b. Clean the gasket, base, and aqueduct by spraying with 70% ethanol and wiping with a Kimwipe.
    c. Carefully inspect for any glass debris, being sure to remove any glass shards.
    d. Assemble the MERSCOPE Flow Chamber base, gasket, aqueduct, and top **WITHOUT** a MERSCOPE Slide in place.
    e. Twist the top clockwise until it cannot rotate further and the "lock" mark on the top is aligned with the "lock" mark on the base to ensure secure assembly.
    f. Store the assembled MERSCOPE Flow Chamber in a cool, dry, dark place.
3. Perform an INSTRUMENT WASH (MAINTENANCE) procedure to as described in Section 9.
4. Repeat the wash program **1x** more (**2x** total).
5. During the 2nd round of washing, check the flow rate as described in Section 9, Step 7.
6. After the last wash, leave the Fluidic Line Connector in place, remove and store the MERSCOPE Wash Cartridge, and close the flow chamber lid and imaging cartridge lid.
7. As applicable, shut down the Instrument Computer and power down the Control Box (this will also shut down the MERSCOPE Imaging Box).

a. **DO NOT** shut down the Analysis Computer until all image processing analysis is complete.

11.2 MERSCOPE Ultra Instrument Restart After Idle Period

1. As applicable, switch on the Control Box (this will also start up the MERSCOPE Ultra Instrument Computer).
   a. Ensure the Analysis Computer is switched on.
2. Follow the instructions in the INSTRUMENT WASH (MAINTENANCE) procedure to complete a wash program.
3. Repeat the wash program **1x** more (**2x** total).
4. During the second program of water cleaning cycle, determine if the flow rate is acceptable as described in Section 9, Step 7.

11.3 MERSCOPE Ultra Instrument Restart After Extended Shutdown

In the case that the instrument has not been used for >3 months, contact Vizgen Support (support@vizgen.com) for advice on instrument startup operations.

12 MERSCOPE ULTRA INSTRUMENT OUTPUT FILE STRUCTURE AND FORMATS

One of the primary outputs of a MERFISH experiment is a list of the spatial locations of RNA molecules in the sample. The RNA molecules need to be pooled together into one or many gene expression profile(s) that describe biological entities. The MERSCOPE Ultra Instrument Software generates single-cell gene expression profiles. To facilitate downstream analysis of MERSCOPE gene expression data, the analysis software provides organized experimental data in open formats (JSON, PNG, CSV, TIFF, PARQUET) and a MERSCOPE Vizualizer file (VZG2) that can be used to explore these outputs in a single interface.

MERSCOPE Ultra also performs downstream analyses leveraging standard Scanpy processes used for the analysis of spatial transcriptomics data. These steps include filtering by transcripts per cell (minimum of 10) and genes per cell (minimum of 1), followed by normalization, log1p transformation, scaling, PCA, UMAP, Leiden clustering, differential expression analysis, and neighboring analysis. The results from these analyses are stored in several files: {experiment_name}.h5ad, cell_categories.csv, cell_numeric_categories.csv, and differentially_expressed_genes.csv.

MERSCOPE Ultra includes compression of raw images captured by the system to maximize storage efficiency. Compressed images are in the format JPEG2000 (JP2).

A summary table of all output files and approximate file size can be found at the end of this section.

12.1 Analysis Output Folder Content

Analysis results of each MERFISH experiment are in a subfolder of Z:\merfish_output\ with the same name as the experiment. In each Z:\merfish_output\{experiment_name}\ folder are:

- experiment.json

- report_{experiment_name}.html
- .png images
- region_{region_name} folders for each individual region:
  - Region Output Folder Content – Always Available
  - Region Output Folder Content – Segmentation Dependent
  - Region Output Folder Content – Images

The **experiment.json** file contains metadata about the experiment: how data were collected and a summary of the analysis outputs. The top-level keys are:

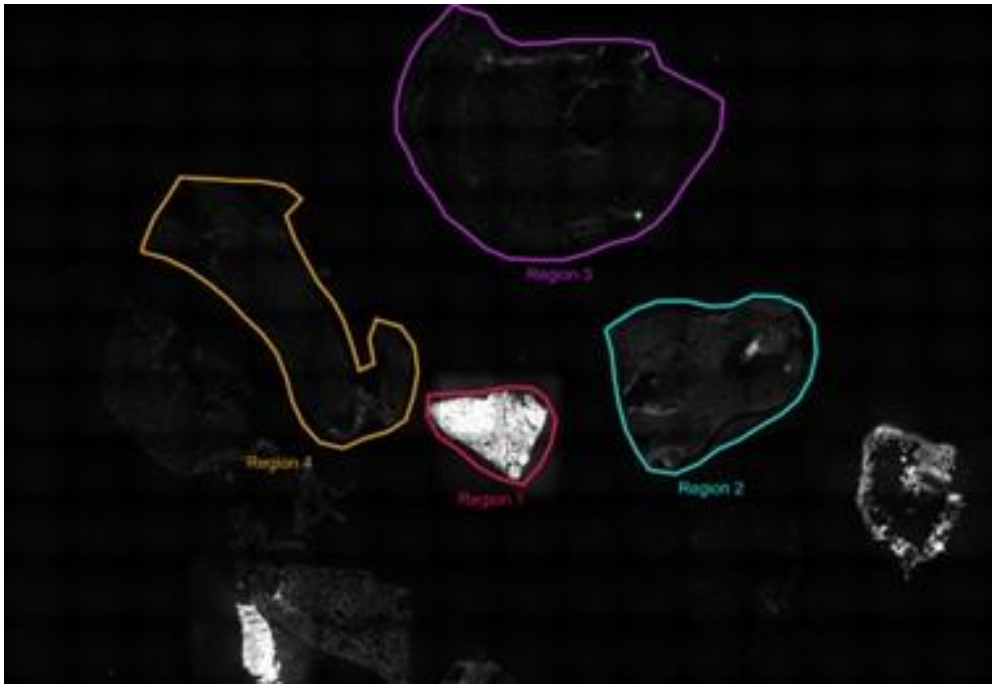
- startDateTime
- userId
- experimentId
- experimentName
- experimentDescription
- experimentDirectory
- codebookName
- codebookId
- panelName
- sampleThickness
- additionalStains
- fiducialColor
- usedReadouts
- usedHybridizationBuffers
- cartridgeBarcode
- regionSummaries
- measurementStartDateTime
- endDateTime
- fovCount
- runMode
- cell_metrics

If segmentation was selected as part of analysis (Segmentation Parameters and Image Processing), the **experiment.json** file contains a per-region summary of the segmentation results and single-cell gene expression, which is saved in the cell_metrics key. cell_metrics contains a list with one object per region. Within each list item there are two keys: region_id, which is the index of the region (see below), and metrics, which contain the following metrics:

num_cells	The number of segmented cells in this region.
volume_stats	The distribution of cell volumes (μm^3).
transcript_count_stats	The distribution of transcripts per cell (count).
ratio_covered	The fraction of the total experimental space covered by a cell.
ratio_transcripts_within_cell	The fraction of all transcripts within a cell.
num_cells_with_no_transcripts	The number of segmented cells that do not contain any transcripts.
enrichment	The per-gene fraction of transcripts within a cell.
sum_signals_medians	The per-channel median of image intensity within a cell, for all imaging channels that are stitched into a mosaic tiff image (Region Output Folder Content – Images). This value is calculated for both the raw image intensity and the high-pass filtered intensity.

report_{experiment_name}.html (MERSCOPE Analysis Report) provides a summary of the segmentation and analysis results for the given experiment in one interactive document. The report includes general experiment information and detailed statistics for transcripts, cells, and auxiliary stains. Tooltips are included throughout the report to provide additional information on key data.

PNG images are derived from low-magnification objective imaging at the beginning of the experiment and named according to the dye. For example, dapi.png for DAPI. The location of each region imaged with the high-magnification objective and analyzed as part of the experiment are overlaid on the PNG images.



Each **region_{region_name}** folder contains all the independently analyzed outputs for each individual region selected for imaging (Select Regions of Interest). Users may have defined unique names for individual regions during experiment setup (Select Regions of Interest) and output files are organized into folders maintaining the user-specified naming.

The relationship between each region index and the name of the region is also saved in the **experiment.json** file under the regionSummaries key. In the example above, the region names in the low magnification mosaic above correspond to the regionSummaries below:

```
"regionSummaries": [
    {
        "name": "Tumor ROI1",
        "startIndex": 0,
        "endIndex": 299
    },
    {
        "name": "Tumor ROI2",
        "startIndex": 300,
        "endIndex": 384
    },
    {
        "name": "TME ROI1",
        "startIndex": 385,
        "endIndex": 645
    },
    {
        "name": "TME ROI2",
        "startIndex": 646,
        "endIndex": 1199
    }
]
```


In this example, the data for the regions would be saved as follows in the table below.

Region Name	Region Index	Output Subfolder
Tumor ROI1	0	region_Tumor ROI1
Tumor ROI2	1	region_Tumor ROI2
TME ROI1	2	region_TME ROI1
TME ROI2	3	region_TME_ROI2

12.2 Region Output Folder Content – Standard

In every region output folder are:

- {experiment_name}.h5ad
- {experiment_name}_region_{region_name}.vzg2
- detected_transcripts.csv

- summary.png

The **{experiment_name}.h5ad** file is generated for use with the Scanpy Single-Cell Analysis pipeline,and its data structure aligns with the guidelines provided in the Scanpy tutorial (see official Scanpy website for additional details). The following fields are contained within the file:

Field	Description
adata.obs['center_x'], adata.obs['center_y']	These fields store the coordinates of cells in micrometer units.
adata.obs['n_count']	Represents the total number of transcripts detected in each cell.
adata.obs['n_genes']	Indicates the total number of unique genes identified in each cell.
adata.var['mean'], adata.var['std']	These fields contain the average and standard deviation of transcript counts for each gene across all cells.
adata.obsm['X_pca']	PCA representation of the data, providing principal component scores for each cell.
adata.uns['pca']['variance_ratio']	This field holds the ratio of explained variance by each principal component.
adata.uns['pca']['variance']	Stores the explained variance (similar to eigenvalues of the covariance matrix) for the PCA analysis.
adata.obsm['X_umap']	Contains UMAP coordinates for the cells, offering a non-linear dimensionality reduction representation.
adata.uns['umap']	Stores parameters used for the UMAP computation.
adata.obs['leiden']	An array with dimensions equal to the number of samples, recording the subgroup ID (e.g., '0', '1', etc.) assigned to each cell during clustering.
adata.uns['leiden']	A dictionary containing the parameters used for the Leiden clustering algorithm, such as resolution, random_state, and number of iterations.
adata.obsp['distances']	This matrix contains the distances from each cell to its nearest neighbors, as identified by the nearest neighbors search.
adata.obsp['connectivities']	A weighted adjacency matrix representing the neighborhood graph of the data points.
adata.uns['neighbors']	This field details the parameters associated with the nearest neighbors computation.

The **{experiment_name}_region_{region_index}.vzg2** file can be opened with the MERSCOPE Vizualizer (Desktop Version). It contains all the information needed to visualize the transcript locations, cell boundaries, and a compressed version of the mosaic image channels (e.g., DAPI, PolyT, Cellbound stains, other additional stains). Refer to the *MERSCOPE Vizualizer User Guide (Desktop Version)* for more information, available online at https://vizgen.com/.

The **detected_transcripts.csv** file is a standard comma separated values (CSV) formatted text file. Column headers:

[BLANK]	A numeric index that uniquely identifies a transcript within a field of view. The index is non-consecutive and ascending within each field of view.
barcode_id	The row index of the identified transcript "barcode" in the codebook file (zero indexed). fov and barcode_id are a composite primary key for the detected_ transcripts.csv table.
global_x	The x-coordinate of the transcript (µm), relative to the space of the experimental region. global_x may be negative in some circumstances depending on the alignment between fields of view.
global_y	The y-coordinate of the transcript (µm), relative to the space of the experimental region. global_y may be negative in some circumstances depending on the alignment between fields of view.
global_z	The index of the z-position. The position is a zero-indexed integer. global_z can be translated into microns using the entry in the first row of the zPos column of the dataorganization.csv file sorted in ascending order.
x	The x-coordinate of the transcript (µm), within the coordinate space of the field of view in which it was imaged.
y	The y-coordinate of the transcript (µm), within the coordinate space of the field of view in which it was imaged.
fov	The index of the field of view in which the transcript was imaged (zero indexed). fov and barcode_id are a composite primary key for the detected_transcripts. csv table.
gene	The human readable name of the gene this transcript is associated with. Gene is derived from the "name" column of the codebook file.
transcript_id	A unique identifier of the gene that this transcript is associated with. transcript_ id is derived from the "id" column of the codebook file.
cell.id	If cell segmentation was performed: The numeric index of the cell that contains this transcript, if any. If this transcript is not associated with a cell, cell_id will be -1. cell_id maps to the EntityID field found in the cell_boundaries.parquet and cell_metadata.csv.

The **summary.png** file is a visual summary of the analysis:

- The spatial distribution of transcripts both within the region and within the mean field of view.
- The "scale factor" used to normalize the brightness of each MERFISH bit before decoding.
- The abundance versus misidentification rate of MERFISH decoding for the experiment.
- Statistics about segmentation and example images of cell boundaries if segmentation was performed.

12.3 Region Output Folder Content – Segmentation Dependent

If segmentation was selected as part of analysis (Segmentation Parameters and Image Processing), files capturing the segmentation include:

- cell_boundaries.parquet
- cell_by_gene.csv
- cell_categories.csv
- cell_metadata.csv
- cell_numeric_categories.csv

- differentially_expressed_genes.csv

The **cell_boundaries.parquet** is in an open- source file format for tabular data that has efficient I/O performance and small file size compared with csv/text files (https://parquet.apache.org/). The **cell_boundaries.parquet** file contains the boundaries of cells in microns, formatted as a data table using GeoPandas. Columns:

[BLANK]	A numeric index. It is sorted and unique, but entries are not consecutive.
ID	A numeric row index that begins from zero. ID is unique within an analysis region.
EntityID	An integer (int64) identifier for a cell or other biological entity identified through spatial analysis. EntityID has the format: analysis timestamp, task index, tile index, geometry index. EntityID is guaranteed to be unique to a biological entity (e.g., cell) within an analysis region. The use of the analysis timestamp in the ID makes the EntityID likely to be unique across all experiments run on a MERSCOPE Instrument. Filtering by EntityID will enable users to get all ZLevels of a given biological entity (e.g., cell).
Name	A free-text description of the geometry in the row.
Type	The type of the entity referred to in EntityID.
ZIndex	The z-index of this slice of the biological entity in the 3D stack, corresponds to global_z in detected_transcripts.csv.
ZLevel	The z-position of this slice of the biological entity in the 3D stack (µm).
Geometry	A valid WKT-format MultiPolygon that describes the biological entity (e.g., cell) at the given ZLevel. The vertices of the MultiPolygon are in microns relative to the space of the experimental region. Geometry uses MultiPolygon objects to describe biologicals entities that may be contiguous in 3D space, but discontiguous at a given ZLevel (i.e., a U-shaped cell). Even if the cell region is contiguous and can be described with a single Polygon, it is stored as a MultiPolygon for data-type consistency.
ParentID	If this biological entity (e.g., cell) is related to a higher-level ("parent") entity, the EntityID of the parent may be stored here. If this biological entity (e.g., cell) does not descend from a parent entity, this value is None.
ParentType	The type of the parent entity, if any. If there is no parent entity, the value is None.

The **cell_by_gene.csv** file is a standard CSV formatted text file. The first column of the file has the header "cell" and is a list of EntityID values corresponding to those in the cell_boundaries. parquet and cell_metadata.csv files. The remaining columns are gene names that correspond with the gene column of the detected_transcripts.csv and the name column of the codebook.

- **IF** the cell_by_gene.csv file is generated by the MERSCOPE Instrument Software, it contains all the same genes as the codebook in the same order, even if no transcripts of a given gene are detected.
- **IF** the cell_by_gene.csv is generated by a post-processing tool that does not have access to the codebook, the number and order of genes in the file may vary.

The **cell_metadata.csv** file is a standard CSV formatted text file. Working with the raw geometry

information for an entire experiment can require significant time and memory resources. Cell metadata is calculated and provided to accelerate some types of geometric operations, such as cell filtering. Entity metadata files have the following columns:

EntityID	These IDs correspond with those in the cell_boundaries.parquet and cell_by_gene.csv files.
fov	The field of view index of the cell or None if the fov information is not available.
volume	The approximate volume of the cell (μm^3). Based on a linear interpolation of each ZLevel of the cell geometries to produce a 3D solid.
center_x	The x-position of the center of the cell in the global coordinate system (µm).
center_y	The y-position of the center of the cell in the global coordinate system (µm).
min_x	The minimum x-extent of the cell (considering all ZLevels) in the global coordinate system (µm).
max_x	The maximum x-extent of the cell (considering all ZLevels) in the global coordinate system (µm).
min_y	The minimum y-extent of the cell (considering all ZLevels) in the global coordinate system (µm).
max_y	The maximum y-extent of the cell (considering all ZLevels) in the global coordinate system (µm).
anisotropy	The ratio of the length of the major axis of the cell to the length of its minor axis (always greater than or equal to 1). A value of 1 represents a circular or square cell.
transcript_count	The number of transcripts, including Blanks, that fall within the cell.
perimeter_area_ratio	The ratio of the perimeter of the cell to its area, calculated at each ZLevel and averaged across occupied ZLevels. Higher values correspond with more complex / non-convex shapes.
solidity	The ratio of the area of the cell to the area of a convex hull around occupied ZLevels, calculated at each ZLevel and averaged across the cell. Lower values correspond with more complex / non-convex shapes.

Furthermore, additional columns are appended for all imaging channels stitched into a mosaic tiff image (Region Output Folder Content – Images). For every cell, the total intensity (for all occupied ZLevels) of each channel within the cell boundary is calculated on the raw fluorescent image and on a high-pass filtered version of the image. These will appear as columns:

- {stain name}_raw
- {stain name}_high_pass

The number of appended columns and naming depends on the configuration of each experiment.

Stain metrics can be helpful for filtering and sorting cells within an experiment. It is not recommended to compare stain metrics between experiments. Many experimental factors, including tissue clarity and image post-processing settings, may affect the values.

12.4 Region Output Folder Content – Images

The **images** subfolder contains images of the experimental region suitable for quantitative analysis and image metadata:

- tiff images
- micron_to_mosaic_pixel_transform.csv
- manifest.json

Every image channel acquired during a MERFISH experiment that is not decoded as a MERFISH bit will be outputted as a **mosaic tiff image**. This includes DAPI, PolyT, Cellbound stains (if applicable), and subsequent round stains (if applicable). Raw data images from the MERFISH experiment are stitched together based on the alignment of fiducial signals to create a mosaic that minimizes the appearance of seams between fields of view. The images are single channel, single plane, 16-bit grayscale tiff files, with the naming convention mosaic_{stain name}_z{ZIndex}.tif

The **micron_to_mosaic_pixel_transform.csv** file is a space-delimited text file with no headers. To overlay transcript locations or cell geometries on the mosaic images, it is necessary to convert coordinates from microns to pixels. The contents of the file are 3 rows of 3 floating point numbers that may be read as a 3 x 3 matrix. This data is an affine transformation matrix describing translation and scaling from microns to pixels. The contents of the file have the form:

Scaling x	0	Translation x
0	Scaling y	Translation y
0	0	1

The x and y translations may be either positive or negative. The origin (0,0) of the pixel coordinate system is not necessarily in the same location as the origin of the micron coordinate system.

The **manifest.json** file contains metadata about the assembly of the mosaic images. The manifest.json file is not necessary for typical downstream analysis but is necessary for rebuilding some analysis outputs.

12.5 MERSCOPE Ultra Instrument Output File Summary

Output File	Description	Typical Size
dapi.png, polyt.png	Images from low-magnification objective, named by dye.	~10 MB
experiment.json	Metadata describing the experiment.	~50 KB
report_{EXPERIMENT_NAME}.html	One-page HTML report with QC stats and analysis overview.	~200 MB
{EXPERIMENT_NAME}.h5ad	AnnData object with data matrices and analysis results.	~0.5-2 GB
{EXPERIMENT_NAME}.vzg2	Same as VZG file, with updated compression.	~5-50 GB
cell_boundaries.parquet	Cell boundaries in microns formatted with GeoPandas.	~0.5-2.5 GB
cell_by_gene.csv	Gene expression matrix by cell.	~50-750 MB
cell_categories.csv	Categorical data for clustered cell groups.	< 1 MB
cell_metadata.csv	Comprehensive metadata for each cell.	~10-250 MB
cell_numeric_categories.csv	Numeric classifications for cells.	~1-40 MB
detected_transcripts.csv	Transcripts detected per cell in CSV and Parquet formats for efficiency.	~1-150 GB
detected_transcripts.parquet	Same as above, detailed transcript data in Parquet format.	~1-30 GB
differentially_expressed_genes.csv	Significant gene expression differences across cell groups with statistics.	< 1 MB
images/mosaic_{stain}_z{[0-9]}.tif	Images and metadata for quantitative analysis of the experimental region. The number of images per experiment depends on run configuration, e.g. inclusion of cell boundary staining.	~10-20 GB per image ~10-100 GB per experiment
micron_to_mosaic_pixel_transform.csv	Transformation parameters from microns to mosaic pixels.	< 1 KB
manifest.json	Metadata for the mosaic image assembly.	< 1 KB
summary.png	Visual summary of the analysis.	< 1 MB
	Estimated Total	**20-260 GB**

13 TECHNICAL TIPS

13.1 Experimental Planning

The MERSCOPE Ultra Instrument analyzes one sample at a time and imaging time is dependent

upon the size of sample, number of genes, and auxiliary staining performed. With the FCX-S (Standard) flow chamber, imaging time ranges between 18-36 hours and, with the FCX-L (Large) flow chamber, imaging time ranges between 34-66 hours.

Sample preparation can be performed in batches and samples can be stored in Clearing Solution/Clearing Premix at 37°C. Refer to the applicable sample preparation user guide for more information, available online at https://vizgen.com/.

13.2 RNase Decontamination

MERFISH measurements are sensitive to RNase activity. RNase contamination of any materials or reagents will degrade data quality.

Samples should be prepared in an area decontaminated with RNaseZap solution.

It is recommended to use RNase-free disposables, e.g., RNase-free media bottles (VWR PN: 82051-594) for preparing buffers.

13.3 MERSCOPE Slide Handling

MERSCOPE Slides are fragile, handle with care. MERSCOPE Slides should be handled with tweezers, positioned to minimize the potential of touching the sample.

13.4 MERSCOPE Flow Chamber Component Cleaning and Storage

Refer to Load – MERSCOPE Flow Chamber for flow chamber component cleaning operations as part of normal operations.

Refer to Clean and MERSCOPE Flow Chamber Idle Procedure for flow chamber cleaning operations in preparation for an idle period.

13.5 MERSCOPE Codebooks

Gene panel-specific MERSCOPE Codebooks are CSV files that can be imported from local storage or the Vizgen Cloud. The applicable MERSCOPE Codebook must imported prior to initiating an experiment.

The instrument scans the MERSCOPE Imaging Cartridge barcode for compatibility with the selected MERSCOPE Codebook after inserting the activated imaging cartridge into the instrument.

14 SAFETY

Safe laboratory practices should be followed at all times.

Discard used MERSCOPE Slides per applicable institutional hazardous waste procedures.

The MERSCOPE Imaging Cartridge contains hazardous material and should be discarded per the MERSCOPE Imaging Cartridge Safety Data Sheet and applicable institutional hazardous waste procedures.

The MERSCOPE Ultra Instrument waste container contents should also be discarded per the MERSCOPE Imaging Cartridge Safety Data Sheet and applicable institutional hazardous waste procedures.

Safety Data Sheets for Vizgen Materials are available online at https://vizgen.com/.

15 FRONT PANEL LED INDICATORS

15.1 MERSCOPE Ultra Imaging Box Front Panel LED Indicator

When the instrument is on, the instrument status is displayed on the MERSCOPE Imaging Box front panel LED indicator.

Green	Progressing	Maintenance or status check running
Green	Constant on	Ready for use, no operations running
Green	Constant on	Configuring the experiment
White	Ramping	Experiment running
White	Breathing	Experiment completed
Blue	Breathing	Post-acquisition processing is complete, and no other processes are running
Red	Constant on	Not ready for use, but no immediate user attention needed
Red	Blinking	Major error requiring user attention (e.g., experiment error, power went off, program error)

15.2 Waste Container LED Indicator

Green	Constant on	Waste container status OK
Red	Blinking	Waste container needs to be emptied

16 ERRORS AND WARNINGS

16.1 Cannot Import the Codebook

This error is received if the MERSCOPE Codebook file name is not in the correct file name format (*codebook_0_panelname_panelID.csv*). Update the file name or select a different file and try again.

This error is received if the MERSCOPE Codebook file name extension is something other than .csv. Only files with .csv extension are allowed. Update the file name extension or select a different file and try again.

This error is received if no genes are detected in the MERSCOPE Codebook file. It is not possible to import a file with 0 genes. Update the file or select a different file and try again.

16.2 Failure to Read the Barcode

This error is received if there is liquid on the barcode on the MERSCOPE Imaging Cartridge and/or the barcode reader in the MERSCOPE Imaging Box. Remove the imaging cartridge from the instrument, dry the barcode and the barcode reader, and reinsert the imaging cartridge. Click Scan Barcode to try barcode scanning again. If the instrument is unable to read the barcode, the barcode number may be entered manually.

16.3 The Barcode Was Not Recognized

This error is received if the MERSCOPE Cartridge has been used already. It is necessary to insert a new imaging cartridge.

This error is received if there is an error in entering the barcode manually. Do not include any dashes or spaces in the number.

This error is received if the instrument software is out of date. Update the software per the instructions.

16.4 The Inserted Imaging Cartridge Does Not Match the Codebook

This error is received if the selected MERSCOPE Codebook requires more bits than available in the inserted MERSCOPE Imaging Cartridge. If the codebook is correct but the imaging cartridge is incompatible, thaw and activate a new imaging cartridge.

16.5 The Imaging Cartridge Has Expired

This error is received if the MERSCOPE Imaging Cartridge inserted has expired. It is possible to proceed, however the experiment may be compromised.

16.6 High-Magnification Objective Focusing Quality is Insufficient

This error is received if the immersion oil on the high-magnification objective is in insufficient quantity or there are air bubbles in the immersion oil. Click **Try Again** to clean the high-magnification objective and repeat the immersion oil application. If the error persists, contact Vizgen Support (support@vizgen.com).

16.7 Update Failed

This error is received if a software update fails. It is possible to try again or try to update the software later.

16.8 Low Liquid Flow Rate Detected When Washing Fluidic Lines

This error is received if the wash fluid is low. If the error persists, contact Vizgen Support (support@vizgen.com).

16.9 Waste Container Not Empty

This error is received if the waste container is too full (overweight). Best practice is to empty the waste container after each wash cycle or before starting an experiment.

16.10 Out of Disk Space

This error is received if there is insufficient disk space on the MERSCOPE Ultra Instrument Computer and/or MERSCOPE Ultra Analysis Computer to run/process an experiment. It is necessary to delete/ remove old experiment files to proceed with a new experiment/image processing analysis.

16.11 Connection Lost

This error is received if the MERSCOPE Ultra Instrument Computer loses connection with the MERSCOPE Ultra Analysis Computer. It will not be possible to run an experiment until the connection is restored. Check the connections. If the error persists, contact Vizgen Support (support@vizgen.com).

This error is received if there is a problem with the hardware on the instrument. If the error persists,

contact Vizgen Support (support@vizgen.com).

Although power cycling the MERSCOPE Ultra Instrument and the MERSCOPE Ultra Analysis Computer may help with restoring connections, **DO NOT** power cycle the MERSCOPE Ultra Analysis Computer while image processing analysis is in process.

17 IN-SOFTWARE OPERATING SYSTEM WARNINGS

Windows Update Settings

This warning will be displayed if Windows Update is not set to Disabled. To clear this warning, disable automatic updates using the Group Policy Editor. Open the Run dialog (Windows key + R) and type gpedit.msc. Navigate to Computer Configuration > Administrative Templates > Windows Components > Windows Update. Double-click Configure Automatic Updates. Select "Disabled" and click Apply, then OK.

17.1 Ethernet Network Adapter Settings

This warning will be displayed if the instrument PC is allowed to disconnect power for connected devices. To clear this warning, open the Control Panel and navigate to Network and Internet. View Network Connections and ensure that no connections are allowed to deactivate by going to Properties and clicking on the Power Management tab. Uncheck the box that says, "Allow the computer to turn off this device to save power," and click OK.

17.2 USB Settings (Power Saving & Suspend)

This warning will be displayed if the instrument PC is allowed to suspend activities of USB devices. To clear this warning, open the Control Panel. Go to System and Security and then Power Options. Click on "Change plan settings" next to your chosen power plan, and then select Change advanced power settings. Under USB Settings, expand the options and change the USB selective suspend setting to Disabled. Click Apply, and then OK.

17.3 Windows Power Plan Settings

This warning will be displayed if the instrument PC is allowed to Hibernate or Sleep. To clear this warning, open the Control Panel. Go to System and Security and then Power Options. Click on "Change plan settings" next to your chosen power plan, and then select Change advanced power settings. Under Sleep, expand the options and change the Sleep after setting to Never. Click Apply, and then OK.

17.4 PCI Express Settings

This warning will be displayed if the instrument PC is allowed to manage the power state of PCI Express devices. To clear this warning, open the Control Panel. Go to System and Security and then Power Options. Click on "Change plan settings" next to your chosen power plan, and then select Change advanced power settings. Under PCI Express, expand the options and change the Link State Power Management setting to Off. Click Apply, and then OK.

18 TROUBLESHOOTING

18.1 Air Bubbles Remain in the MERSCOPE Flow Chamber After Wetting

It is possible to pull more liquid to try and remove air bubbles. The user interface leads users through this process. It is only possible to pull more liquid twice.

If air bubbles are still visible after pulling more liquid once, ensure the fluid connections are correctly assembled and tightly closed before pulling liquid for a second time.

18.2 Air Bubbles Remain in the MERSCOPE Flow Chamber After 2x Wetting

Contact Vizgen Support (support@vizgen.com). If the air bubbles are small, it may be possible to proceed with an experiment. However, the experiment may be compromised.

18.3 Air Bubbles in the MERSCOPE Flow Chamber Input Line After Priming

If the input line is not debubbled at least up to the Fluidic Line Connector during priming, which may be a consequence of a clogged fluidic line or a faulty MERSCOPE Imaging Cartridge, contact Vizgen Support (support@vizgen.com).

18.4 Incompletely Thawed MERSCOPE Imaging Cartridge

It is essential to fully thaw the MERSCOPE Imaging Cartridge prior to activation, layering with mineral oil, and inserting into the instrument. An incompletely thawed imaging cartridge will not perform as expected and the experiment and/or the instrument may be compromised.

18.5 MERSCOPE Imaging Cartridge Not Activated Before Insertion into the MERSCOPE Ultra Instrument

It is highly recommended to activate the MERSCOPE Imaging Cartridge prior to insertion in the instrument. However, if forgotten, it is possible to pierce the Cartridge Activate Port, insert the Imaging Activation Mix, and then layer the mineral oil while the imaging cartridge is in the instrument. If the imaging cartridge lid has been closed before activation, other reagent access points in the imaging cartridge foil will be punctured (by the imaging cartridge lid) – ensure no material/liquid is dropped into these other reagent access points.

DO NOT invert the imaging cartridge after the activation port has been punctured and/or the imaging cartridge lid has been closed.

18.6 MERSCOPE Imaging Cartridge Not Activated Before Initiating an Experiment

It is essential to activate the MERSCOPE Imaging Cartridge (and layer mineral oil) prior to initiating an experiment. If users forget to activate the imaging cartridge, the experiment will fail. The experiment should be aborted if users realize they have forgotten to activate the imaging cartridge.

18.7 Mineral Oil Not Layered into a MERSCOPE Imaging Cartridge Before Barcode Reading

It is highly recommended to layer the mineral oil into the MERSCOPE Imaging Cartridge prior to insertion in the instrument. However, if forgotten, it is possible to layer the mineral oil while the

activated imaging cartridge is in the instrument (as long the user has not proceeded to barcode reading, at which point the experiment is initiated and the imaging cartridge lid is locked). If the imaging cartridge lid has been closed before activation, other reagent access points in the imaging cartridge foil will be punctured (by the imaging cartridge lid) – ensure no material/liquid is dropped into these other reagent access points. **DO NOT** invert the imaging cartridge after the activation port has been punctured and/or the imaging cartridge lid has been closed.

18.8 Mineral Oil Not Layered into an Activated MERSCOPE Imaging Cartridge Before Initiating an Experiment

It is essential to layer mineral oil into the activated MERSCOPE Imaging Cartridge prior to initiating an experiment. If users forget to layer mineral oil, they may proceed but the experiment may be compromised.

18.9 MERSCOPE Imaging Cartridge Thawed but Activation and Experiment Start is Delayed

It is highly recommended to work with a MERSCOPE Imaging Cartridge directly after thawing. Contact Vizgen Support (support@vizgen.com) if the imaging cartridge is thawed but an experiment cannot be started for some time.

18.10 Acquire Focus with High-magnification Objective Fails

If the instrument is unable to return an error during Acquire Focus with the high-magnification objective, the software will walk the user through cleaning the objective and reapplying immersion oil. It is also important to note that the underside of the MERSCOPE Slide must be cleaned prior to loading the flow chamber into the system. Liquid, dust, or debris on the bottom surface of the slide will compromise focus quality. The bottom surface of the MERSCOPE Slide should be carefully but thoroughly cleaned with 70% ethanol as described in this user manual and the software user interface.

18.11 MERSCOPE Flow Chamber Aqueduct Caught in Base When Trying to Disassemble

If the Aqueduct of the Flow Chamber sticks when trying to remove it from the Base, ensure the Top is removed and then place the Base, MERSCOPE Slide, Gasket, and Aqueduct in a container with nuclease-free water up to the level where the Aqueduct meets the Base, and then place the container in ultrasonic water bath for 5 min before trying to remove the Aqueduct.

18.12 Abort Experiment

If the experiment must be aborted, click **Abort Experiment** in the top right-hand corner. If the experiment is aborted, all data will be corrupted, and the information will be lost. Reasons users may need to abort an experiment include:

- Realization that experimental setup was incorrect.
- Realization that sample preparation was incorrect.
- Realization that the MERSCOPE Imaging Cartridge was not activated prior to insertion in the instrument.

After aborting an experiment, contact Vizgen Support (support@vizgen.com) prior to removing the MERSCOPE Imaging Cartridge for advice on cleaning the instrument.

The MERSCOPE Imaging Cartridge may not be able to be reused after aborting an experiment. Contact Vizgen Support (support@vizgen.com) for advice.

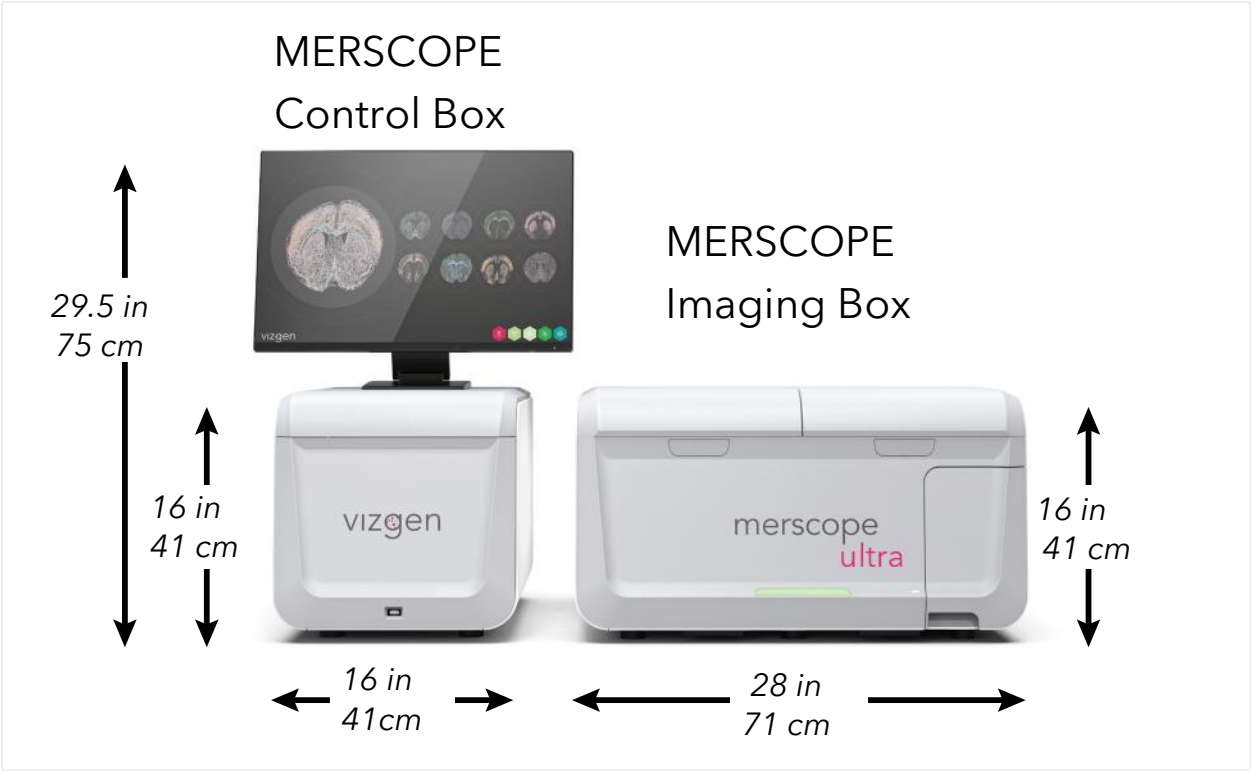
19 APPENDIX I: SAFETY DOCUMENTATION

19.1 Intended Use of Equipment

The MERSCOPE Ultra Instrument is intended for research use only. The instrument enables massively multiplexed, error-robust, single-cell in situ transcriptomic imaging. The MERSCOPE Ultra Instrument integrates high-resolution imaging, fluidics, and image processing into automated hardware to deliver precise measurements.

19.2 Technical Specification

Feature	Specification
Vizgen Flow Chambers Supported	FCX-S (Standard): 1.25 cm^2 of tissue per experiment FCX-L (Large): 3 cm^2 of tissue per experiment
Throughput	Up to 9.0 cm^2 per week
Optical Resolution	Oil immersion; High numerical aperture objective
Lateral Resolution	100 nm pixel size
Transcript Localization Precision (X and Y)	<20 nm
Slide Capacity	Single MERSCOPE slide
Multiplexing Capacity	Up to 1000 plex
Imaging Camera	Back-thinned cooled sCMOS camera
Illumination	Multi-color laser
On-Instrument Data Storage Capacity	58 TB
Analysis PC Storage Capacity	58 TB
Long-Term Data Storage	Customer-provided long-term storage
Instrument Dimensions	See figures below
Automated Image Processing	Transcript decoding and cell segmentation


MERSCOPE
Control Box
MERSCOPE
Imaging Box
29.5 in
75 cm
16 in
41 cm
16 in
41 cm
16 in
41cm
28 in
71 cm
vizgen
merscope
ultra


MERSCOPE Ultra
Control Box
MERSCOPE Ultra
Imaging Box
29 in
74 cm
vizgen
merscope
ultra

19.3 Name and Address of the Manufacturer or Supplier from Whom Technical Assistance May Be Obtained

Vizgen Inc.
61 Moulton Street
Cambridge MA,
USA, 02138

19.4 Information to Mitigate Risks Found in The Risk Assessment

Regarding the risks of using the MERSCOPE Ultra Instrument, there are no user-serviceable components, subsystems inside the system. All services shall be conducted by qualified Vizgen Service Engineers.

19.5 Instructions for Lifting and Carrying

Only authorized Vizgen representatives should uncrate and install the MERSCOPE Ultra Instrument. Mishandling of the instrument can affect the alignment or damage instrument components.

Do not relocate the instrument after installation and preparation. Moving the instrument improperly can affect optical alignment and compromise data integrity. If the instrument must be relocated, contact Vizgen Support (support@vizgen.com).

Uncrating or moving an instrument by anyone other than an authorized Vizgen representative will void the warranty.

19.6 Equipment Ratings

19.6.1 MERSCOPE Ultra Instrument Supply Voltage and Power Requirements

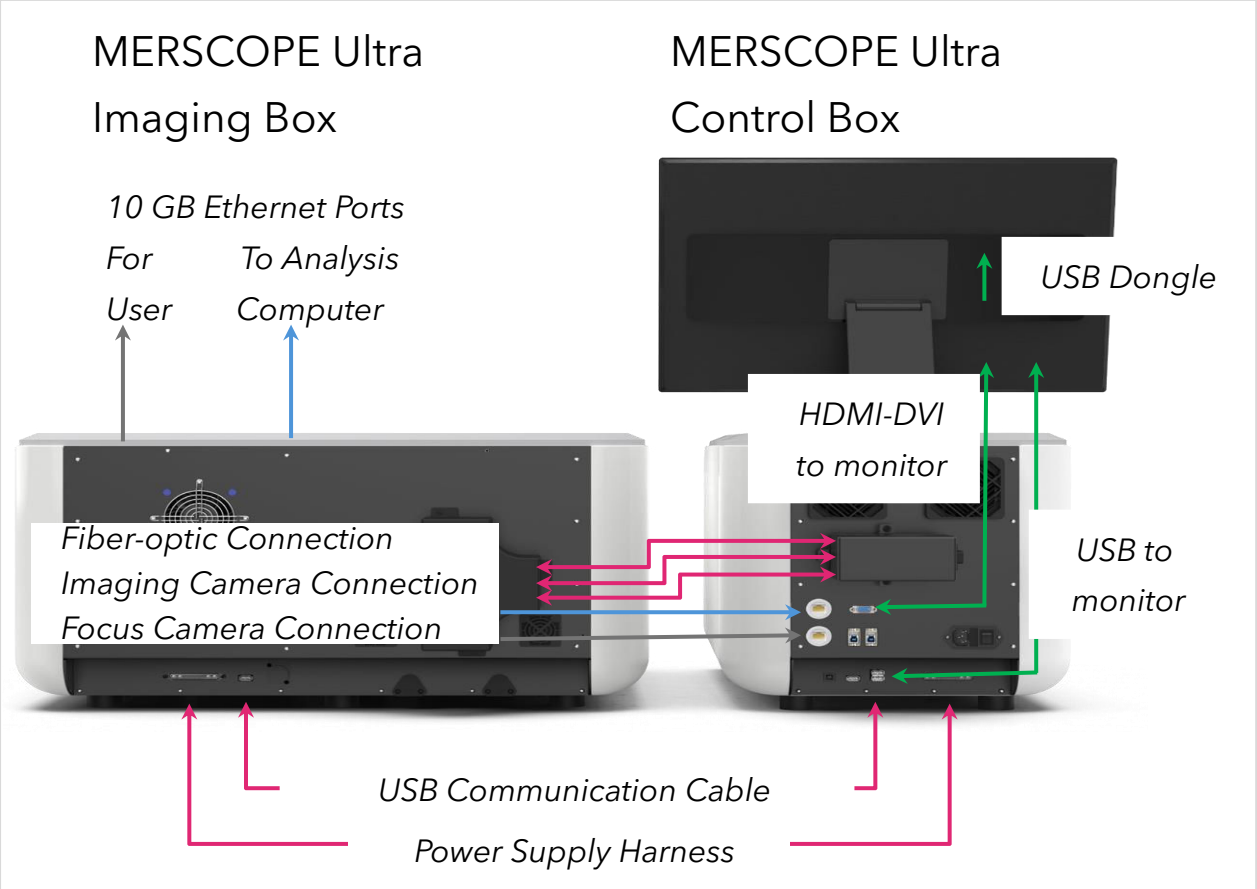
A maximum of 1650 W is required for the MERSCOPE Ultra Instrument, MERSCOPE Ultra Analysis Computer, and the instrument monitor.

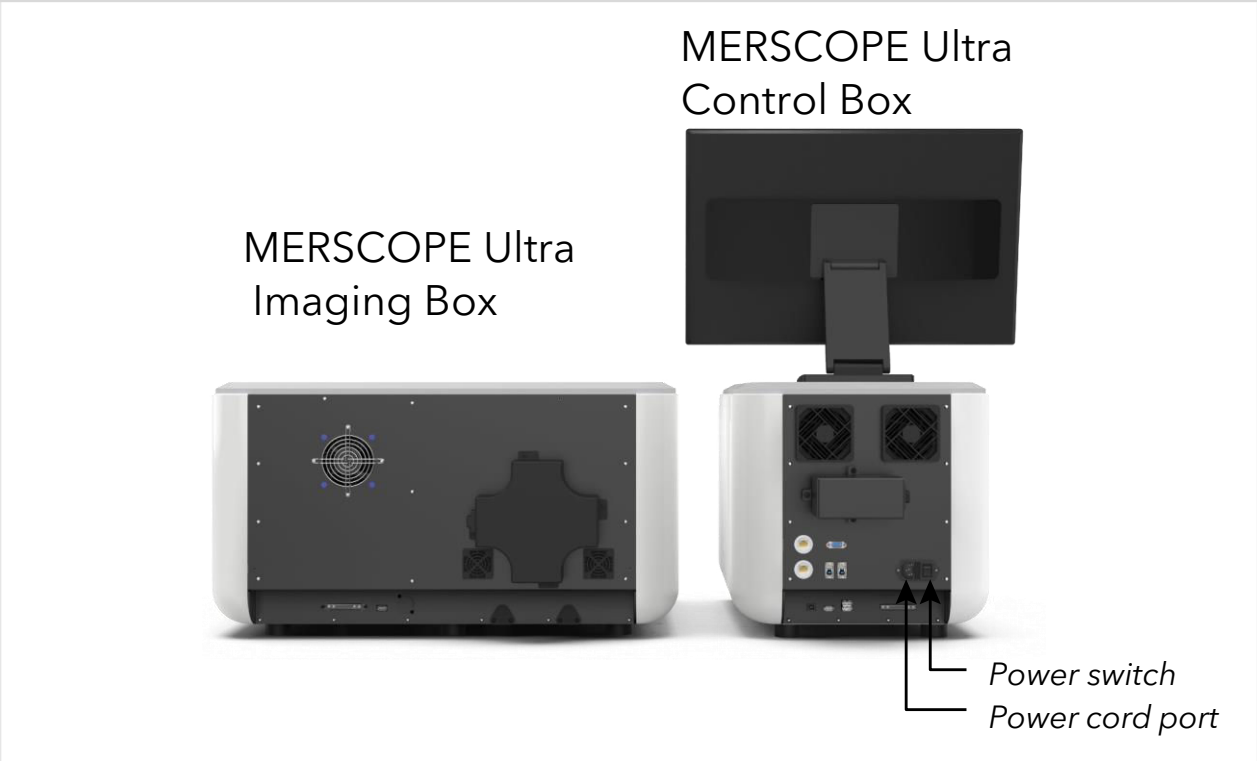
Instrument Power Requirements	Specification
Line Voltage	100-240 VAC – 50/60 Hz
Power Supply Rating	650 W

Analysis Computer Power Requirements	Specification
Line Voltage	100-240 VAC – 50/60 Hz
Power Supply Rating	1000 W

Monitor Requirements	Specification
Line Voltage	100-240 VAC – 50/60 Hz
Power Supply Rating	28 W Typical (<1 W standby)

19.6.2 A Description of All Input and Output Connections





19.6.3 Environmental Conditions

Element	Specification
Operating Environment	Indoor use only.
Operation Altitude	2000 meters above sea level.
Temperature	Maintain a laboratory temperature of 19°C to 25°C. During a run, do not allow the ambient temperature to exceed 32°C.
Humidity	Maintain a non-condensing relative humidity between 20 and 80%.
Air Quality	Keep the instrument away from sources of dust. For indoor use only.
Vibration	Avoid intermittent shocks or disturbances near the instrument.

A user-supplied uninterruptible power supply (UPS) is highly recommended. Vizgen is not responsible for runs affected by interrupted power regardless of whether the MERSCOPE Ultra Instrument and MERSCOPE Ultra Analysis Computer are connected to a UPS. Standard generator- backed power is often not uninterruptible, and a brief power outage is typical before power resumes.

Consult with an institutional facility manager to obtain a UPS that complies with local standards.

Specification	Japan	North America	International
Description	APC Smart-UPS 2200 LCD 100 V (Japan)	APC Smart-UPS 2200VA, Tower, LCD 120 V with SmartConnect Port	APC Smart-UPS 2200VA, Tower, LCD 230 V with SmartConnect Port
Part Number	SMT2200J	SMT2200C	SMT2200IC
Maximum Output Capacity	1.98 kWatts / 2.2 kVA	1.92 kWatts / 1.92 kVA	1.98 kWatts / 2.2 kVA
Input Voltage (nominal)	100 VAC	120 VAC	230 VAC
Input Frequency	50/60 Hz	50/60 Hz	50/60 Hz
Input Connection	NEMA L5-30P	NEMA 5-20P	IEC 320 C20, Schuko CEE 7 / EU1-16P
Dimensions (H × W × D)	17 × 8 × 22 in 43 × 20 × 56 cm	17 × 8 × 22 in 43 × 20 × 56 cm	17 × 8 × 22 in 43 × 20 × 56 cm
Weight	123 lb 56 kg	112 lb 51 kg	110 lb 50 kg
Typical Run Time (1200 W)	Approx. 17 min	Approx. 20 min	Approx. 19 min

The instrument is equipped with an international standard IEC 60320 C14 receptacle and is shipped with a region-specific power cord. Hazardous voltages are removed from the instrument only when the power cord is disconnected from the AC power source. To obtain equivalent receptacles or power cords that comply with local standards, contact Vizgen Support (support@vizgen.com).

19.7 Equipment Installation

For MERSCOPE Ultra Instrument installation and operation requirements, review the *MERSCOPE Ultra Instrument Site Preparation Guide*, Document Number 91500102. Vizgen Service Engineers should refer to the *MERSCOPE Ultra Instrument Installation Guide*.

19.8 Equipment Operation

See previous sections of the *MERSCOPE Ultra Instrument User Guide* for equipment operation instructions. For replacement of consumable materials, contact Vizgen Support (support@vizgen.com). For legal notices regarding the MERSCOPE Ultra Instrument, see page 2 of the *MERSCOPE Ultra Instrument User Guide*.

19.9 Equipment Maintenance and Service

1. Refer to this manual and other documents provided by Vizgen on the proper usage and routine maintenance of the MERSCOPE Ultra Instrument. For instrument preventative maintenance and service, contact Vizgen Support (support@vizgen.com). Vizgen Service Engineers should refer to the *MERSCOPE Ultra Instrument Service Guide*.
2. The instrument requires the use of the power cord supplied by Vizgen during the initial installation process.
3. The instrument does not require your on-site service personnel to maintain or service. All services shall be conducted by qualified Vizgen Service Engineers.