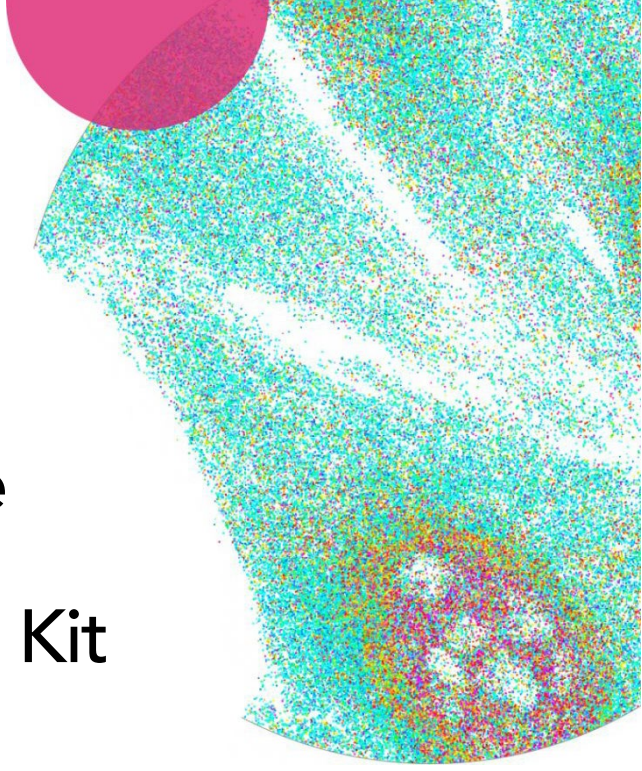
91600103 Rev A



MERSCOPE™ User Guide

Protein Stain Verification Kit

Vizgen™ Materials*

10400106 - 10400111	MERSCOPE Protein Stain Kits, 20 samples *To detect user-provided primary antibodies raised in mouse, rabbit, goat, rat, human, and chicken*
10400112	MERSCOPE Protein Stain Verification Kit (Mouse, Rabbit, Goat), 5 samples
10400113	MERSCOPE Protein Stain Verification Kit (Rat, Human, Chicken), 5 samples

**In addition to applicable MERSCOPE sample preparation kits. No MERSCOPE Imaging Kits are required for protein stain verification.*

vizgen

NOTICES

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Document revision	Rev A
Revision date	September 2022

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SUPPORT

Contact information

support@vizgen.com

61 Moulton Street

West Cambridge Science Park

Cambridge, MA 02138

USA

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

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PROTEIN STAIN VERIFICATION OVERVIEW

General

This user guide is intended to guide users through a protocol to verify that their (user-provided) primary antibodies are compatible with MERSCOPE Protein Stain Kits, and that sample preparation will result in adequate protein imaging quality with the MERSCOPE Instrument. The verification protocol includes a rapid imaging protocol (~1 h) and does not require the use of a MERSCOPE Imaging Cartridge. This user guide is generally **applicable to fresh frozen and paraformaldehyde (PFA)-fixed frozen tissue and cultured cells**. It is not applicable to formalin-fixed paraffin-embedded (FFPE) tissue. Vizgen supports mouse and human tissues samples only.

Protein stain verification is based on immunofluorescence staining. The MERSCOPE Protein Stain Kits use oligonucleotide-conjugated secondary antibodies to detect user-provided primary antibodies raised in mouse, rabbit, goat, rat, human, and chicken.

User-provided primary antibodies **MUST** be:

- Bovine serum albumin (BSA)-free, **AND**
- Compatible with immunohistochemistry (IHC).

The MERSCOPE Protein Stain Verification Kits enable **concurrent** verification of primary antibodies raised in:

- Mouse, rabbit, goat, **OR**
- Rat, human, chicken.

DAPI and PolyT staining are also included in the Protein Stain Verification Reagent. It is **NOT** possible to use both MERSCOPE Protein Stain Verification Kits on the same sample at the same time.

NO cell boundary staining should be performed during protein stain verification.

The protein stain encoding probes (conjugated to secondary antibodies) have been assigned with unique readout bits and can be imaged in a single round with laser channels 749, 647, and 561.

This user guide is complementary to Vizgen's sample preparation user guides. Users should start by reading the applicable sample preparation user guide. Several steps in the sample preparation user guides are replaced with steps outlined in this user guide. This user guide also leads users through protein stain verification imaging.

For general MERSCOPE Instrument information and guidance (including troubleshooting, errors), refer to the *MERSCOPE Instrument User Guide*, available online at https://vizgen.com/.

Sample Preparation Variables

Vizgen's sample preparation user guides outline protocols for sample preparation, including protein staining. The MERSCOPE Protein Stain Verification Kits enable users to verify their (user-provided) primary antibodies are compatible with MERSCOPE Protein Stain Kits, and that sample preparation will result in adequate protein imaging quality with the MERSCOPE Instrument. Furthermore, different sample types may require optimization of certain steps or conditions and/or users may wish to incorporate their own sample preparation conditions. The variables that commonly affect sample RNA quality, protein stain, and imaging quality are:

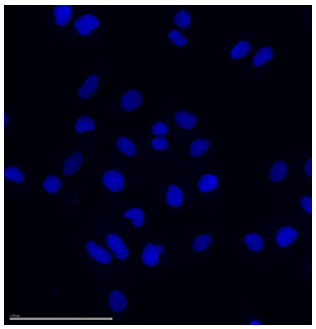
- Initial sample protein quality
- User-provided primary antibody quality
- Working concentration of use-provided primary antibody*
- Presence of BSA in the primary antibody solution
- Autofluorescence quenching requirements and duration
- Fixation reagents and duration
- Permeabilization reagents and duration
- Gel embedding quality
- Digestion requirement and duration (as part of clearing)
- Clearing duration and temperature
- Tissue sectioning technique
- Cultured cell concentrations

*Primary antibody concentration may first be determined by regular immunofluorescence staining and then further optimized using the verification workflow.

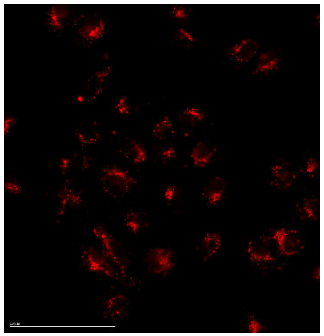
Protein Stain Verification Examples – U2OS Cell Line

U2OS Cell Line

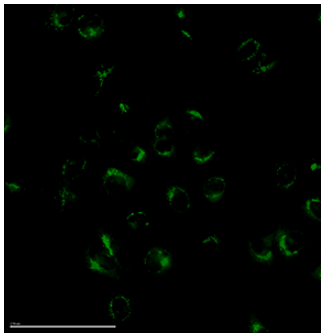
Target protein	Primary antibody raised in	Protein Stain included in Secondary Staining Solution	Protein Stain Verification Reagent
LAMP1	Mouse	Anti-Mouse Aux 4 Protein Stain	Protein Stain Verification Reagent (Mouse, Rabbit, Goat)
GORASP2	Rabbit	Anti-Rabbit Aux 5 Protein Stain	
SOX9	Goat	Anti-Goat Aux 6 Protein Stain	



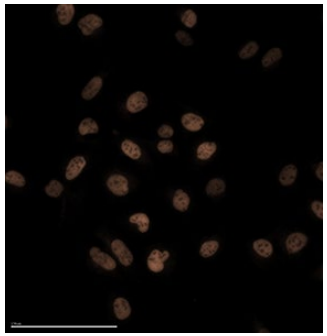
DAPI



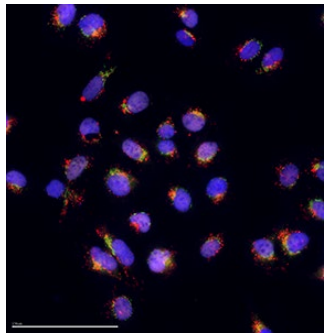
Mouse LAMP1



Rabbit GORASP2



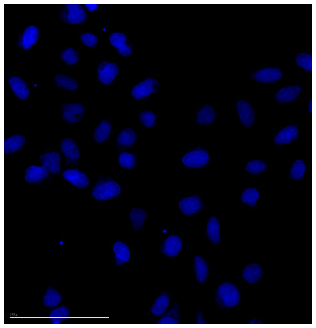
Goat SOX9



Merged with DAPI

U2OS Cell Line

Target protein	Primary antibody raised in	Protein Stain included in Secondary Staining Solution	Protein Stain Verification Reagent
Tubulin	Rat	Anti-Rat Aux 7 Protein Stain	Protein Stain Verification Reagent (Rat, Human, Chicken)
Actin	Human	Anti-Human Aux 8 Protein Stain	
HSP60	Chicken	Anti-Chicken Aux 9 Protein Stain	



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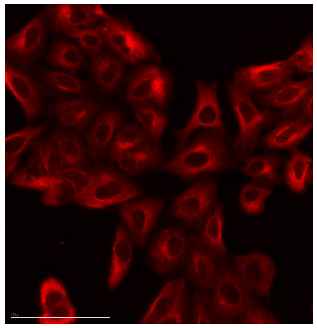
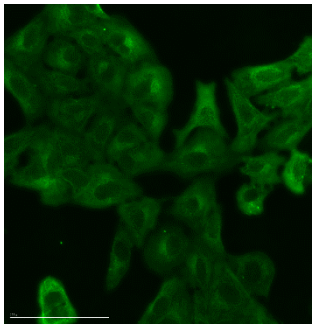
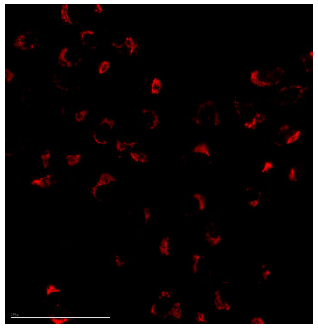


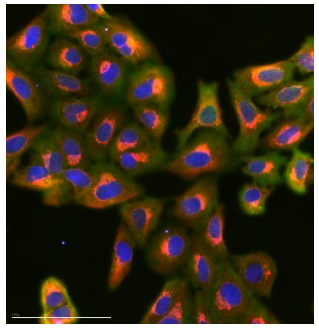
Rat Tubulin



Human Actin



Chicken HSP60

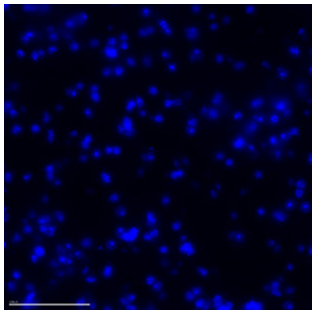


Merged with DAPI

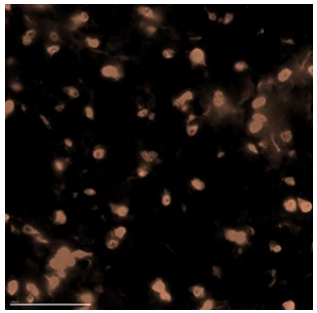
Protein Stain Verification Examples – Mouse Brain

Mouse Brain

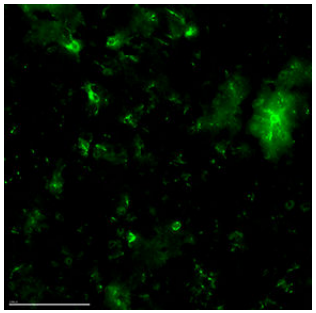
Target protein	Primary antibody raised in	Protein Stain included in Secondary Staining Solution	Protein Stain Verification Reagent
NeuN	Mouse	Anti-Mouse Aux 4 Protein Stain	Protein Stain Verification Reagent (Mouse, Rabbit, Goat)
Iba1	Rabbit	Anti-Rabbit Aux 5 Protein Stain	
MOG	Goat	Anti-Goat Aux 6 Protein Stain	



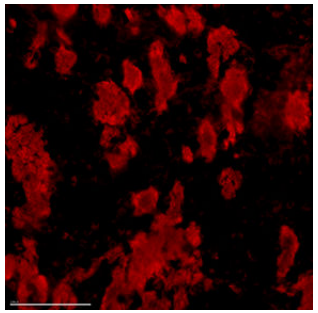
DAPI



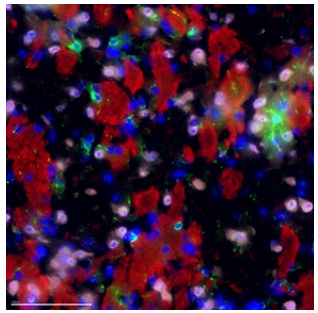
Mouse NeuN



Rabbit Iba1



Goat MOG

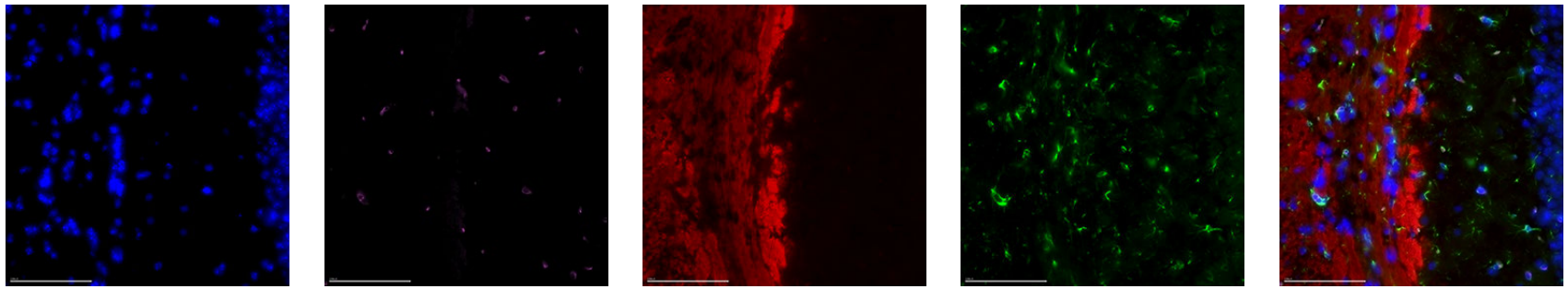


Merged with DAPI

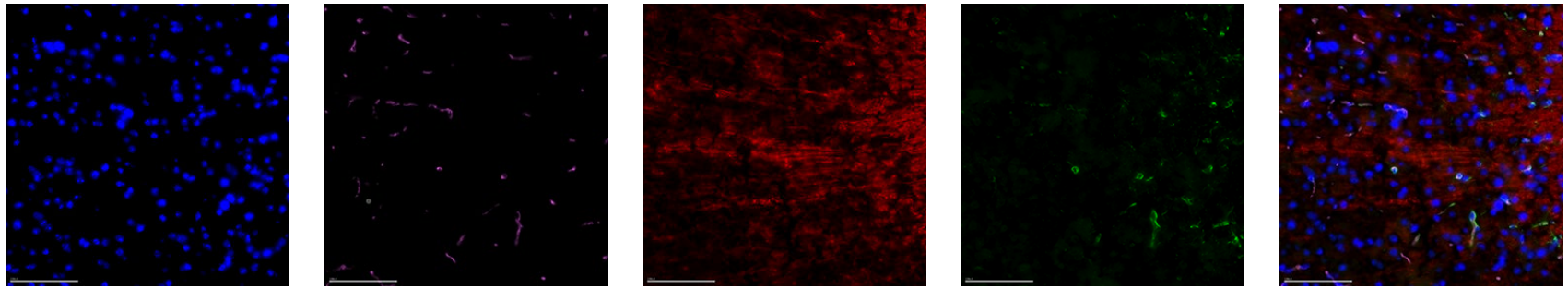
Mouse Brain

Target protein	Primary antibody raised in	Protein Stain included in Secondary Staining Solution	Protein Stain Verification Reagent
CD31	Rat	Anti-Rat Aux 7 Protein Stain	Protein Stain Verification Reagent (Rat, Human, Chicken)
MBP	Human	Anti-Human Aux 8 Protein Stain	
GFAP	Chicken	Anti-Chicken Aux 9 Protein Stain	

Field of view 1



Field of view 2



DAPI **Rat CD31** **Human MBP** **Chicken GFAP** **Merged with DAPI**

Protein Stain Verification Alignment with Sample Preparation & Instrument User Guides

	Frozen Tissue	Cultured Cells	Protein Stain Verification	
Sample Preparation Kit/Guide	Tissue Sectioning & Fixation	Cell onto MERSCOPE Slide and Fixation	Sample-dependent method onto MERSCOPE Slide and Fixation	Protein Stain Verification Kit/Guide
	Permeabilization	Permeabilization	Permeabilization	
	Cell Boundary Staining and/or Protein Staining (both optional)	Cell Boundary Staining and/or Protein Staining (both optional)	**NO** Cell Boundary Staining Protein Staining	
	Encoding Probe Hybridization	Encoding Probe Hybridization	**NO** Encoding Probe Hybridization	
	Post Encoding Probe Wash	Post Encoding Probe Wash	**NO** Post Encoding Probe Wash	
	Gel Embedding	Gel Embedding	Gel Embedding	
	Digestion (Optional)		Digestion (sample dependent)	
	Clearing	Clearing	Clearing	
MERSCOPE Imaging Kit/Instrument Guide	DAPI and PolyT Staining Reagent	DAPI and PolyT Staining Reagent	**Protein Stain Verification** Reagent	
	Insert MERSCOPE Slide into MERSCOPE Flow Chamber	Insert MERSCOPE Slide into MERSCOPE Flow Chamber	Insert MERSCOPE Slide into MERSCOPE Flow Chamber	
	Add Imaging Buffer Activator and **RNase inhibitor** to MERSCOPE Imaging Cartridge	Add Imaging Buffer Activator and **RNase inhibitor** to MERSCOPE Imaging Cartridge	Add Imaging Buffer Activator to Imaging Buffer	
	Insert cartridge into instrument	Insert cartridge into instrument	**Manual** filling of flow chamber	
	Automated fluidics and data acquisition	Automated fluidics and data acquisition	Data acquisition	

MATERIALS

Vizgen Materials – Protein Stain Verification Kits

These materials are **in addition to** Vizgen materials and other materials outlined in the applicable sample preparation user guides.

Use Sample Prep Wash Buffer (PN 20300001) and Formamide Wash Buffer (PN 20300002) from the applicable MERSCOPE sample preparation kit.

MERSCOPE Protein Stain Verification Kit (Mouse, Rabbit, Goat), 5 samples	10400112	Storage
Protein Stain Verification Reagent (Mouse, Rabbit, Goat)	20300109	–20°C
Imaging Buffer	20300016	–20°C
Imaging Buffer Activator	20300015	–20°C
Minimize freeze-thaw cycles		

MERSCOPE Protein Stain Verification Kit (Rat, Human, Chicken), 5 samples	10400113	Storage
Protein Stain Verification Reagent (Rat, Human, Chicken)	20300110	–20°C
Imaging Buffer	20300016	–20°C
Imaging Buffer Activator	20300022	–20°C
Minimize freeze-thaw cycles		

Vizgen Materials – Protein Stain Kits

MERSCOPE Anti-Mouse Protein Stain Kit, 20 samples	10400106	Storage
Blocking Buffer C Premix, 4 x 5 samples*	20300100	–20°C
Anti-Mouse Aux 4 Protein Stain	20300101	–20°C
*Minimize freeze-thaw cycles		

MERSCOPE Anti-Rabbit Protein Stain Kit, 20 samples	10400107	Storage
Blocking Buffer C Premix, 4 x 5 samples*	20300100	–20°C
Anti-Rabbit Aux 5 Protein Stain[a]	20300102	–20°C
*Minimize freeze-thaw cycles [a] Not compatible with the MERSCOPE Cell Boundary Stain Kit		

MERSCOPE Anti-Goat Protein Stain Kit, 20 samples	10400108	Storage
Blocking Buffer C Premix, 4 x 5 samples*	20300100	−20°C
Anti-Goat Aux 6 Protein Stain	20300103	−20°C
*Minimize freeze-thaw cycles		

MERSCOPE Anti-Rat Protein Stain Kit, 20 samples	10400109	Storage
Blocking Buffer C Premix, 4 x 5 samples*	20300100	−20°C
Anti-Rat Aux 7 Protein Stain	20300104	−20°C
*Minimize freeze-thaw cycles		

MERSCOPE Anti-Human Protein Stain Kit, 20 samples	10400110	Storage
Blocking Buffer C Premix, 4 x 5 samples*	20300100	−20°C
Anti-Human Aux 8 Protein Stain	20300105	−20°C
*Minimize freeze-thaw cycles		

MERSCOPE Anti-Chicken Protein Stain Kit, 20 samples	10400111	Storage
Blocking Buffer C Premix, 4 x 5 samples*	20300100	−20°C
Anti-Chicken Aux 9 Protein Stain	20300106	−20°C
*Minimize freeze-thaw cycles		

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

PROTEIN STAIN VERIFICATION BENCH PROTOCOL

Substitute the Protein Staining, Encoding Probe Hybridization, and Post Encoding Probe Hybridization steps in sample preparation user guides with the Protein Stain Verification Staining step outlined below. **NO** cell boundary staining should be performed during protein stain verification.

Fresh and Fixed Frozen Tissue Sample Preparation User Guide Steps	Cultured Cells Sample Preparation User Guide Steps	Protein Stain Verification
I. Tissue Sectioning, Fixation, Permeabilization	I. MERSCOPE Slide Preparation and Fixation	Per applicable sample preparation user guide
-	II. Permeabilization	Per applicable sample preparation user guide
II. Protein Staining ONLY*	III. Protein Staining ONLY*	Instructions below in this user guide
III. Encoding Probe Hybridization	IV. Encoding Probe Hybridization	
IV. Post Encoding Probe Hybridization	V. Post Encoding Probe Hybridization	
V. Gel Embedding	VI. Gel Embedding	Per applicable sample preparation user guide
VI. Clearing	VII. Clearing	Per applicable sample preparation user guide
*__NO__ cell boundary staining should be performed during protein stain verification, and therefore the alternative steps at this point of the protocol are not applicable (i.e., the steps titled "Cell Boundary Staining ONLY" and "Cell Boundary Staining AND Protein Staining" are not applicable to protein stain verification).		

Protein Stain Verification Staining – Kit/Reagent Selection

Select the reagents to be used in protein stain verification based on the host species of the user-provided primary antibodies. It is **NOT** possible to use both MERSCOPE Protein Stain Verification Kits on the same sample at the same time.

User-provided primary antibody raised in	Protein Stain included in Secondary Staining Solution	Applicable Protein Stain Verification Reagent
Mouse	Anti-Mouse Aux 4 Protein Stain	Protein Stain Verification Reagent (Mouse, Rabbit, Goat)
Rabbit	Anti-Rabbit Aux 5 Protein Stain	
Goat	Anti-Goat Aux 6 Protein Stain	
Rat	Anti-Rat Aux 7 Protein Stain	Protein Stain Verification Reagent (Rat, Human, Chicken)
Human	Anti-Human Aux 8 Protein Stain	
Chicken	Anti-Chicken Aux 9 Protein Stain	

Protein Stain Verification Staining

> ***REMEMBER**, in any one sample, primary antibodies can only be verified in host species groups of:*
>
> - *Mouse, rabbit, goat, **OR***
> - *Rat, human, chicken*

> *Maintain user-provided primary antibodies, and MERSCOPE Protein Stains in a benchtop cooler until use. Spin down using a benchtop centrifuge before use.*
>
> *Thaw Blocking Buffer C Premix. Ensure fully thawed and mixed, and spin down using a benchtop centrifuge before use.*
>
> *Return unused reagents to −20°C storage but minimize freeze-thaw cycles.*

1. Aspirate the 70% ethanol. Add **5 mL** 1X PBS.
2. Prepare Blocking Solution:

Blocking Solution	1 sample	5 samples	10 samples
Blocking Buffer C Premix (PN 20300100)	100 µL	500 µL	1 mL

3. Aspirate the 1X PBS to dry the MERSCOPE Slide, leaving just enough liquid to cover the tissue section.
4. Add **100 µL** Blocking Solution onto the center of the tissue section. Use scissors to cut a piece of parafilm 2×2 cm. Use tweezers to peel off the parafilm backing and place the side previously protected by the backing onto the solution. Avoid introducing air bubbles.

> *If the Blocking Solution is not spread across the cells, lift and then lower the parafilm with tweezers until the Blocking Solution is spread across the cells. The parafilm should fit within the MERSCOPE Slide, otherwise the Blocking Solution may wick away into the petri dish.*

5. Incubate at room temperature for 1 h.

6. Prepare Primary Staining Solution:

Primary Staining Solution	1 sample	5 samples	10 samples
Blocking Buffer C Premix (PN 20300100)	100 µL	500 µL	1 mL
EITHER user-provided primary antibody raised in[a-c]: • Mouse • Rabbit • Goat	1 µL of each	5 µL of each	10 µL of each
OR user-provided primary antibody raised in[a-c]: • Rat • Human • Chicken	1 µL of each	5 µL of each	10 µL of each

a. Add the primary antibodies for **EACH** protein to be detected, **based on the available groups**. E.g., in a single sample, if a protein is detected using a mouse primary antibody and a second protein is detected using a goat primary antibody, add the mouse primary antibody **AND** the goat primary antibody to this Primary Staining Solution. Primary antibodies raised in mouse and chicken **CANNOT** be verified in a single sample at the same time, for example.

b. Only 1 primary antibody **PER SPECIES** can be detected in a single MERFISH experiment. **DO NOT** add 2 primary antibodies raised in the same species.

c. Primary antibody concentration(s) may first be determined by regular immunofluorescence staining and then further optimized using this verification workflow.

7. Use tweezers to remove the parafilm.
8. Aspirate the solution to dry the MERSCOPE Slide, leaving just enough liquid to cover the tissue section.
9. Add **100 µL** Primary Staining Solution onto the center of the tissue section. Use scissors to cut a piece of parafilm 2×2 cm. Use tweezers to peel off the parafilm backing and place the side previously protected by the backing onto the solution. Avoid introducing air bubbles.

If the Primary Staining Solution is not spread across the cells, lift and then lower the parafilm with tweezers until the Primary Staining Solution is spread across the cells. The parafilm should fit within the MERSCOPE Slide, otherwise the Primary Staining Solution may wick away into the petri dish.

10. Incubate at room temperature for 1 h.
11. Use tweezers to remove the parafilm.

12. Wash **3x** with **5 mL** 1X PBS, incubate 5 min on a rocker each wash.
13. Prepare Secondary Staining Solution:

Secondary Staining Solution		1 sample	5 samples	10 samples
Blocking Buffer C Premix (PN 20300100)		100 µL	500 µL	1 mL
EITHER Protein Stain(s) Select among[a]:				
Anti-Mouse Aux 4	(PN 20300101)	1 µL of each	5 µL of each	10 µL of each
Anti-Rabbit Aux 5	(PN 20300102)			
Anti-Goat Aux 6	(PN 20300103)			
OR Protein Stain(s) Select among[a]:				
Anti-Rat Aux 7	(PN 20300104)	1 µL of each	5 µL of each	10 µL of each
Anti-Human Aux 8	(PN 20300105)			
Anti-Chicken Aux 9	(PN 20300106)			

a. **ONLY** add the protein stain(s) against **EACH** primary antibody host species used in the Primary Staining Solution, **based on the available groups**. E.g., if mouse and goat primary antibodies were used in the Primary Staining Solution, add the required volume of anti-mouse and anti-goat, protein stain to this Secondary Staining Solution.

14. Aspirate the 1X PBS to dry the MERSCOPE Slide, leaving just enough liquid to cover the tissue section.
15. Add **100 µL** Secondary Staining Solution onto the center of the tissue section. Use scissors to cut a piece of parafilm 2×2 cm. Use tweezers to peel off the parafilm backing and place the side previously protected by the backing onto the solution. Avoid introducing air bubbles.

If the Secondary Staining Solution is not spread across the cells, lift and then lower the parafilm with tweezers until the Secondary Staining Solution is spread across the cells. The parafilm should fit within the MERSCOPE Slide, otherwise the Secondary Staining Solution may wick away into the petri dish.

16. Incubate at room temperature for 1 h.
17. Use tweezers to remove the parafilm.

18. Wash **3x** with **5 mL** 1X PBS, incubate 5 min on a rocker each wash.
19. Aspirate the 1X PBS. In a fume hood, add **5 mL** fixation buffer to fix the stained tissue section at room temperature for 15 min.
20. Wash **2x** with **5 mL** 1X PBS, incubate 5 min each wash.

Continue with the Gel Embedding from the applicable sample preparation user guide. Continue sample preparation through Clearing and clear the samples for at least 24 h before proceeding to protein stain verification imaging.

PROTEIN STAIN VERIFICATION IMAGING SUMMARY

Step	Summary
From Sample Preparation with Verification Probes	➡ Sample is on a MERSCOPE Slide in Clearing Solution
Stain	➡ On the bench, stain the sample on a MERSCOPE Slide with the applicable Protein Stain Verification Reagent
Configure	➡ Start Verification in the MERSCOPE Instrument user interface. Configure the MERSCOPE Instrument with sample details ➡ No Codebook is necessary for protein stain verification
Load Cartridge ***NOT*** *used in verification*	➡ The MERSCOPE Imaging Cartridge from the previous run should remain in place. No imaging cartridge is used in protein stain verification. The imaging cartridge lid should remain closed during protein stain verification.
Load Flow Chamber	➡ Assemble the stained MERSCOPE Slide into the MERSCOPE Flow Chamber ➡ Activate the Imaging Buffer ➡ Manually fill the flow chamber with Activated Imaging Buffer ➡ Connect the filled flow chamber to the MERSCOPE Instrument fluidic lines ➡ Insert the filled flow chamber into the instrument and lock into place
Select	➡ 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 100 mm^2
Switch Objective	➡ Remove the filled MERSCOPE Flow Chamber from the MERSCOPE Instrument WITHOUT detaching the fluidic lines ➡ Apply immersion oil to the high-magnification objective ➡ Re-insert the flow chamber into the instrument and lock into place
Verification	➡ Acquire protein stain verification data ➡ Initiate image processing analysis
Clean	➡ Remove the immersion oil from the high-magnification objective ➡ Prepare the MERSCOPE Instrument for the next sample. **IF** the instrument will idle or only run verification for ≥2 weeks, follow an idle period preparation procedure (refer to the *MERSCOPE Instrument User Guide*)
Transfer	➡ Data may be transferred off the MERSCOPE Instrument after data acquisition is complete (via ethernet port or portable hard drive)

PROTEIN STAIN VERIFICATION IMAGING STEP-BY-STEP

Protein stain verification imaging is different from routine sample imaging. It is **NOT** necessary to use a MERSCOPE Imaging Cartridge for protein stain verification. It is also **NOT** necessary to import a MERSCOPE Codebook for protein stain verification.

Prepare for Protein Stain Verification Imaging

- Take Sample Prep Wash Buffer (PN 20300001) and Formamide Wash Buffer (PN 20300002) from the applicable MERSCOPE sample preparation kit.
- Warm up the **applicable** Protein Stain Verification 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.

Primary antibody raised in	Applicable reagent
• Mouse • Rabbit • Goat	Protein Stain Verification Reagent (Mouse, Rabbit, Goat) (PN 20300109)
• Rat • Human • Chicken	Protein Stain Verification Reagent (Rat, Human, Chicken) (PN 20300110)

- Thaw Imaging Buffer (PN 20300016). Ensure fully thawed and mixed before use.
- Maintain Imaging Buffer Activator (PN 20300015) in a benchtop cooler until use. Spin down using a benchtop centrifuge before use.
- Return unused reagents (stored at −20°C) to −20°C but minimize freeze-thaw cycles.

Stain the MERSCOPE Slide with Protein Stain Verification Reagent

> ***PROTECT FROM LIGHT*** *at all times during staining and after the sample is mounted in the MERSCOPE Flow Chamber, until loaded in the MERSCOPE Instrument.*
>
> *Formamide Wash Buffer is hazardous. Perform these steps in a fume hood.*

- Aspirate the Clearing Solution (from sample preparation), and ensure all Clearing Solution is aspirated from the petri dish.
- Wash **2x** with **5 mL** Sample Prep Wash Buffer.
- On the lowest setting, gently vortex the tube of the **applicable** Protein Stain Verification Reagent tube to ensure the reagent is well mixed and no precipitate is visible.
- Add **3 mL** Protein Stain Verification Reagent, incubate 15 min on a rocker.
- Wash **1x** with **5 mL** Formamide Wash Buffer, incubate 10 min.
- Wash **1x** with **5 mL** Sample Prep Wash Buffer.
- Proceed immediately to the next step.

MERSCOPE Instrument Operations for Protein Stain Verification

Configure the Verification Experiment – Start Verification

➡ Click **Start Verification** on the display and allow 1-2 min for the MERSCOPE Instrument to initialize.

– *Prior to starting a new Verification, there must be at least 5 TB of disk space available on the MERSCOPE Instrument Computer and 5 TB of disk space available on the MERSCOPE Analysis Computer. If insufficient space is available, old datasets should be copied to network storage or a portable USB hard drive.*

➡ Enter experimental details (name, description).

➡ Select verification type **Protein**.

➡ Specify sample thickness. If sample thickness is unknown, 10 µm should be selected.

➡ Select the experimental stain group used. Select [**Anti-Mouse, Anti-Rabbit, Anti-Goat**] if the user-provided antibody was raised in mouse, rabbit, goat. Select [**Anti-Rat, Anti-Human, Anti-Chicken**] if the user-provided primary antibody was raised in rat, human, chicken.

– *The list of channels that appear below when an experimental stain group is selected correspond to the protein stains used in the Secondary Staining Solution during sample preparation.*

➡ Select the illumination intensity **Protein (Bright)**, **Protein (Medium)**, or **Protein (Dim)** for each channel.

– *Select bright, medium, or dim based on the relative abundance of the target proteins in the sample to equilibrate the intensity of the readout. Bright illumination (high laser power) should be selected for proteins with low expression and dim illumination (low laser power) should be selected for proteins with high expression.*

➡ The channels can be renamed by touching the name.

➡ At the end of configuration, a **Verification Configuration** summary will appear.

➡ If the Verification Configuration summary is satisfactory, click **Next**.

– *DAPI and PolyT stains are always imaged automatically.*

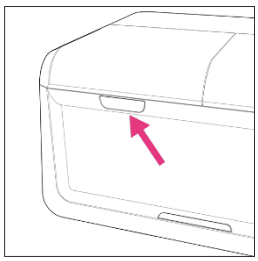
> *It is **NOT** necessary to install a MERSCOPE Imaging Cartridge for protein stain verification.*
>
> *The imaging cartridge from the previous run should remain in place.*
>
> *The imaging cartridge lid should remain closed during protein stain verification.*

Load – MERSCOPE Flow Chamber Preparation for Verification

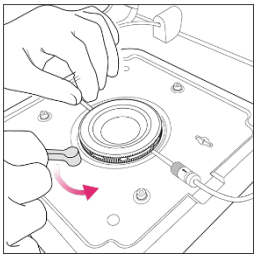
In general, the MERSCOPE Flow Chamber should be lifted out of, and placed into, the stage adapter by gently holding the fluidic lines to either side of the flow chamber and lifting/placing vertically (i.e., do not tilt the Aqueduct).

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

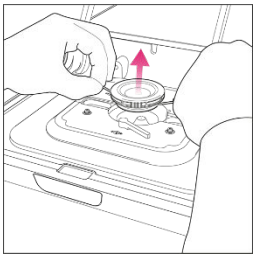
- Open the flow chamber lid, unlock the MERSCOPE Flow Chamber from the previous run, and remove from the stage adapter in the MERSCOPE Instrument. **IF** the flow chamber has been in storage prior to initiating a run, go directly to the flow chamber disassembly and cleaning steps.
- Detach the fluidic lines from the MERSCOPE Instrument.



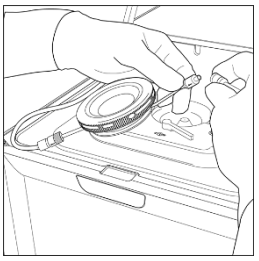
Open the flow chamber lid



Unlock the flow chamber



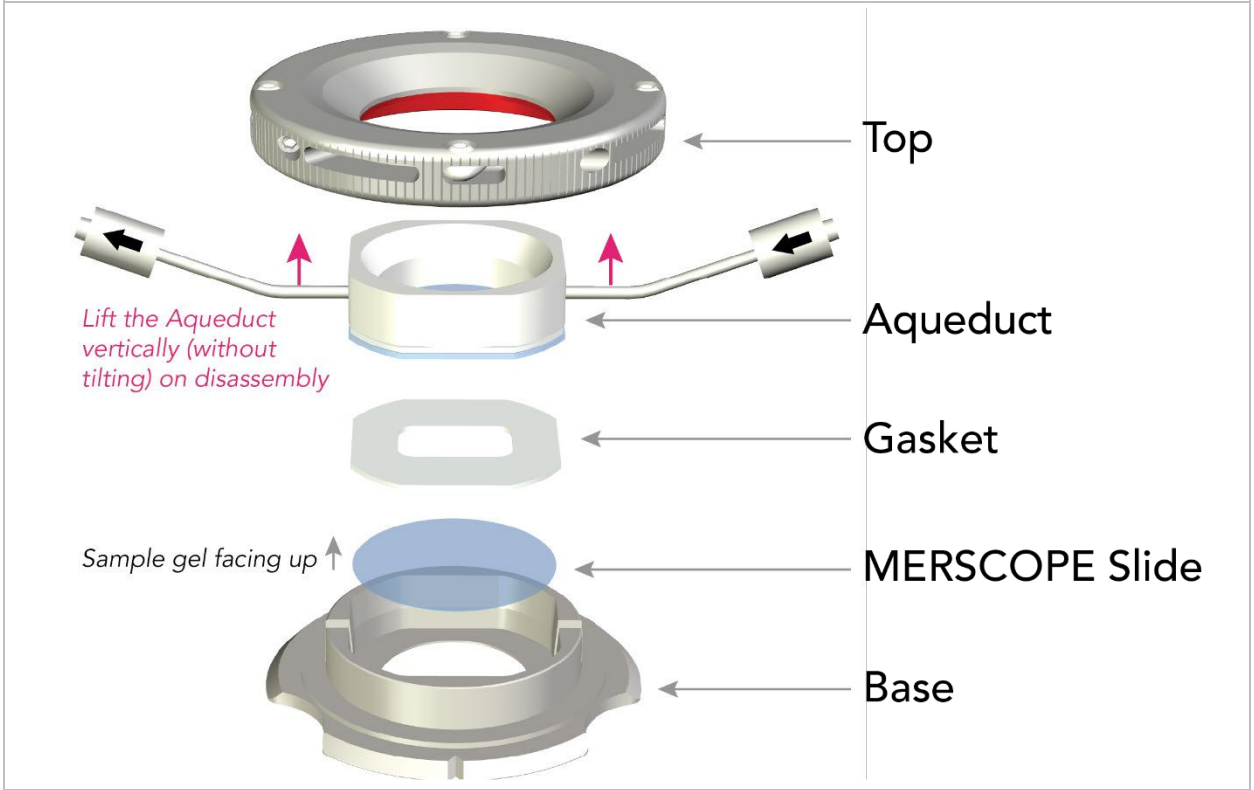
Remove the flow chamber from the stage adapter

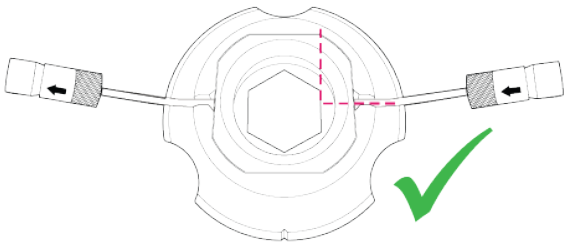


Detach the flow chamber fluidic lines from the instrument

- Rotate the MERSCOPE Flow Chamber Top counterclockwise to disassemble the flow chamber (refer to next pages for images). Discard the previous MERSCOPE Slide per applicable institutional hazardous waste procedures.
- Clean the MERSCOPE 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.
- Hold the MERSCOPE Flow Chamber Base close to the sample petri dish. Gently pick up the MERSCOPE Slide with tweezers or gloved fingers and place into the Base (sample gel facing up).
- Assemble the MERSCOPE Flow Chamber by placing the MERSCOPE Gasket on top of the MERSCOPE Slide (refer to next page for image of correct gasket orientation).
- 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 a loud click is heard to ensure secure assembly.
- Once assembled, spray the bottom of the MERSCOPE Slide with 100% ethanol and wipe clean with a Kimwipe. Repeat **2x** more (3x total) to ensure the optical imaging surface is clean.

MERSCOPE Flow Chamber Part Names and Gasket Orientation





The MERSCOPE Gasket should be orientated with a long side of the hexagon positioned perpendicular to the fluid input line

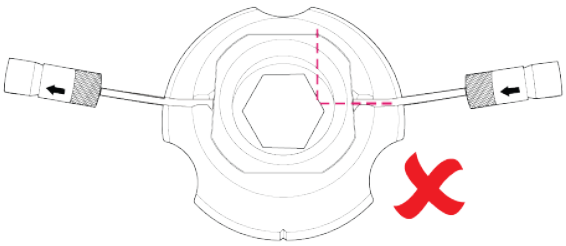
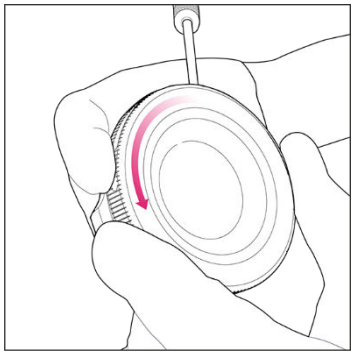
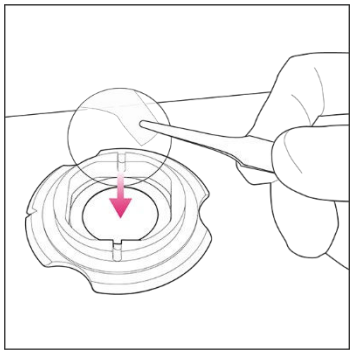


Illustration of an **INCORRECT** orientation of the MERSCOPE Gasket

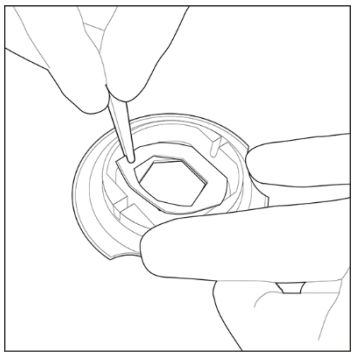
MERSCOPE Flow Chamber Assembly and Cleaning



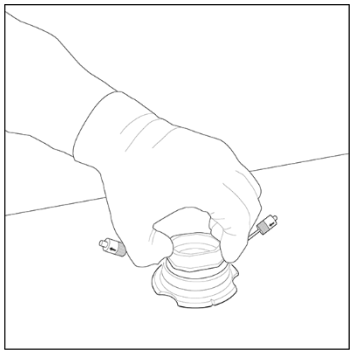
Rotate the Top counterclockwise to disassemble the flow chamber. Clean the Gasket, Base, and Aqueduct



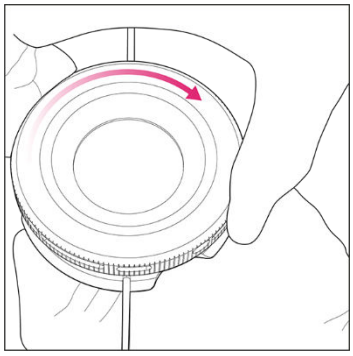
Place the new MERSCOPE Slide in the Base, sample gel facing up



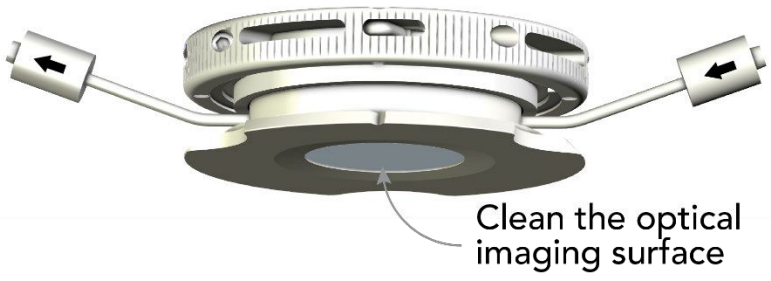
Place the MERSCOPE Gasket on top of the MERSCOPE Slide. Refer to previous page for correct gasket orientation

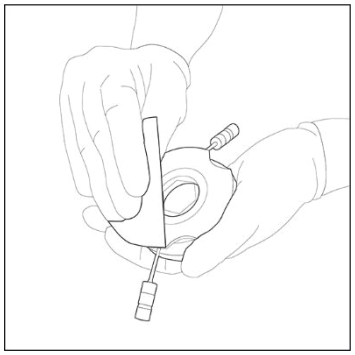


Assemble the Aqueduct. Ensure the notch in the Base and the flow direction arrows marked on the Aqueduct connectors are oriented correctly



Assemble the Top and twist the Top clockwise until a loud click is heard





➡ Activate the Imaging Buffer:

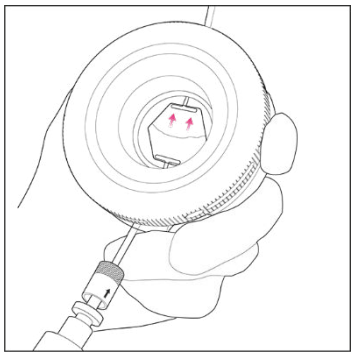
Activated Imaging Buffer	1 sample
Imaging Buffer (PN 20300016)	5 mL
Imaging Buffer Activator (PN 20300015)	25 µL
Gently invert the tube at least 3x to ensure adequate mixing but without introducing air bubbles	

➡ Transfer the Activated Imaging Buffer to a syringe without creating air bubbles.

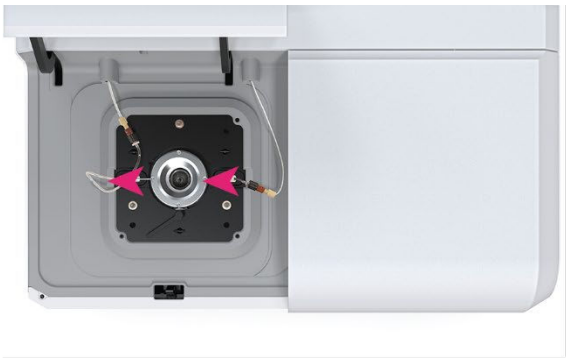
➡ Use the Imaging Fluid Line Adapter (PN 30400010) to connect the syringe to the input line of the assembled MERSCOPE Flow Chamber.

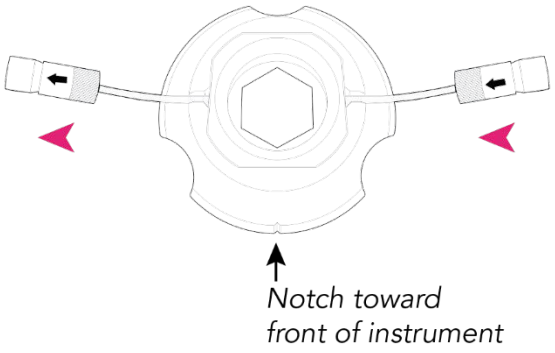
➡ Orient the MERSCOPE Flow Chamber vertically with the output line upwards and collect any fluid coming out of the output line in an appropriate waste container.

➡ Slowly introduce a minimum of 2 mL Activated Imaging Buffer to fill the MERSCOPE Flow Chamber and ensure air bubbles are driven out of the flow chamber and fluidic lines.



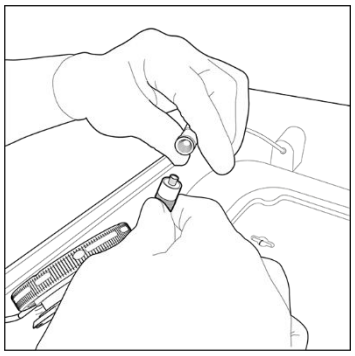
Orient the MERSCOPE Flow Chamber with the output line upwards. Fill the MERSCOPE Flow Chamber with Activated Imaging Buffer via a syringe and Imaging Fluid Line Adapter



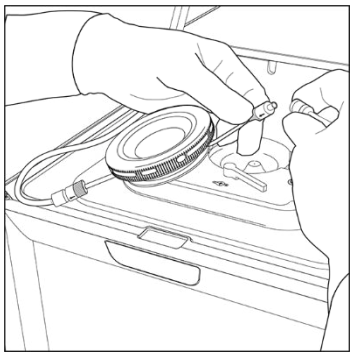


It is critical to insert the MERSCOPE Flow Chamber into the MERSCOPE Instrument in the correct orientation. The Aqueduct has flow direction arrows marked on the connectors (illustrated without the Top in place). The flow chamber should be connected to and inserted into the instrument with the notch in the Base toward the front of the instrument.

➡ Connect the filled MERSCOPE Flow Chamber fluidic lines to those in the MERSCOPE Instrument. First connect the output line (to the left). Then connect the input line (to the right).



Connect the flow chamber output line to the instrument (to the left)



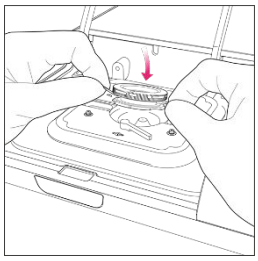
Connect the flow chamber input line to the instrument (to the right)

➡ Insert the filled MERSCOPE Flow Chamber into the MERSCOPE Instrument. First insert the back edge of the flow chamber under the overhangs in the stage adapter. Then, lower the flow chamber to lie flat within the stage adapter.

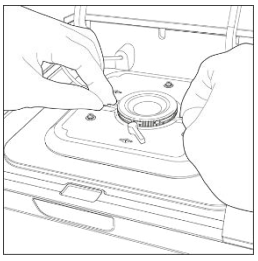
➡ Lock the MERSCOPE Flow Chamber into place.

– *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 the insertion again, ensuring the back edge of the of the flow chamber is first inserted under the overhangs.*

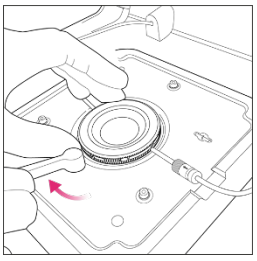
➡ Close the flow chamber lid and click **Acquire Mosaic** to acquire a low-resolution mosaic.



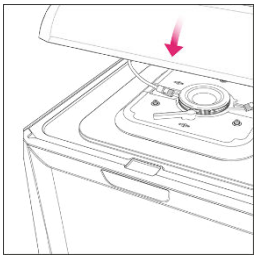
Insert the back edge of the flow chamber under the overhangs in the stage adapter



Lower the flow chamber to lie flat within the stage adapter



Lock the flow chamber into place



Close the flow chamber lid

Select Regions of Interest

This step is done using a low-magnification objective.

– *The MERSCOPE Instrument will acquire a low-resolution mosaic.*

➡ Select the regions of interest to be included in the experiment using the touchscreen or mouse. Draw boundaries on the mosaic to define the region of interest for imaging. Once a boundary is drawn it is saved and a summary appears on the right-hand side of the screen.

➡ Drawing another boundary automatically creates a new region.

➡ 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.

➡ The regions can be renamed at any time by touching the name.

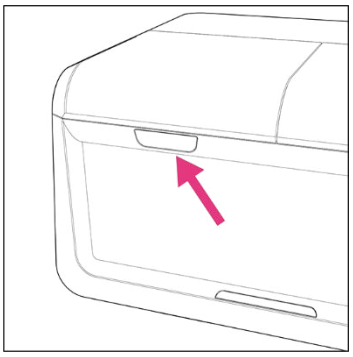
➡ Up to 10 regions can be selected with a **total area of up to 100 mm²** (1 cm^2).

➡ Toggle between **PolyT** and **DAPI** and move the **Visible Intensity Range** slider to adjust the contrast of the image.

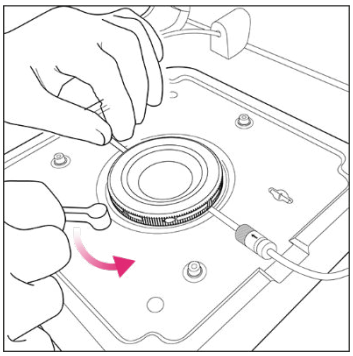
➡ When selections are complete, click **Next**.

Switch to the High-Magnification Objective

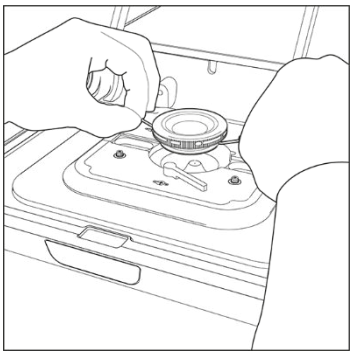
➡ Open the flow chamber lid and unlock and remove the MERSCOPE Flow Chamber from the stage adapter but **DO NOT DETACH** the fluidic lines. Click **Next** to advance through screens as operations are performed.



Open the flow chamber lid



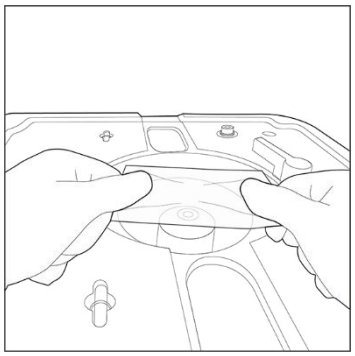
Unlock the flow chamber



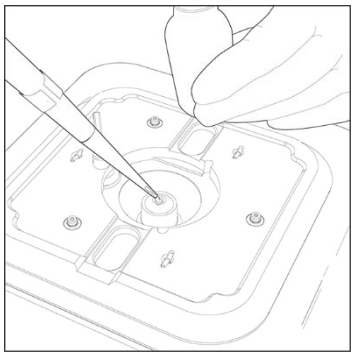
Remove the flow chamber from the stage adapter but DO NOT DETACH the fluidic lines

➡ Clean the immersion oil from the high-magnification objective with lens tissue.

➡ Pipette **50 µL** 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



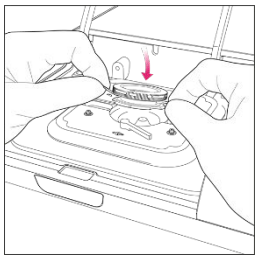
Pipette 50 µL immersion oil onto the high-magnification objective. Check for bubbles in the immersion oil

➡ Re-insert the MERSCOPE Flow Chamber into the MERSCOPE Instrument. First insert the back edge of the flow chamber under the overhangs in the stage adapter. Then, lower the flow chamber to lie flat within the stage adapter.

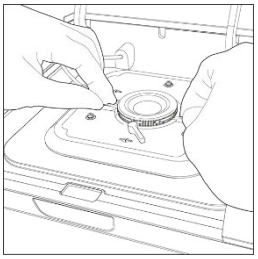
➡ Lock the MERSCOPE Flow Chamber into place.

– *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 the insertion again, ensuring the back edge of the of the flow chamber is first inserted under the overhangs.*

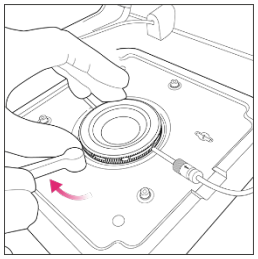
➡ Close the flow chamber lid and click **Acquire Focus**.



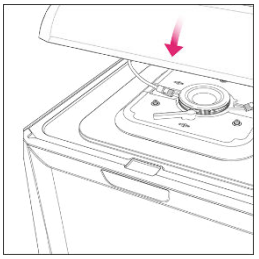
Insert the back edge of the flow chamber under the overhangs in the stage adapter



Lower the flow chamber to lie flat within the stage adapter



Lock the flow chamber into place



Close the flow chamber lid

Verification – Data Acquisition

– *The MERSCOPE Instrument will attempt to find the focal plane with the high-magnification objective.*

➡ If the focusing is successful, click **Next** to advance to a **Verification Experiment Summary**.

> *If the MERSCOPE 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.*

➡ If the **Verification Experiment Summary** is satisfactory, click **Start Verification** to initiate the experiment.

– *The MERSCOPE Instrument will automatically run the sample. A progress bar reports experimental progress.*

– *The MERSCOPE Instrument will indicate* ***Done*** *when all measurements are complete.*

➡ Click **Start Image Processing** to initiate image processing analysis.

– *The MERSCOPE Instrument will automatically copy the raw image data to the MERSCOPE Analysis Computer to begin or queue the image processing analysis.*

– *Image processing analysis will continue in the background after an experiment is completed on the MERSCOPE Instrument.*

➡ Click **Clean Instrument** to proceed to cleaning the MERSCOPE Instrument.

Image Transfer

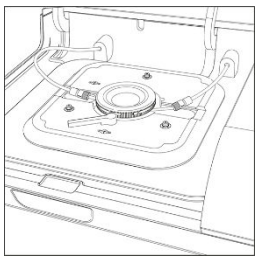
➡ After the experiment is completed, verification data are available on the instrument in D:\MERSCOPEDATA for download and visual inspection.

Clean

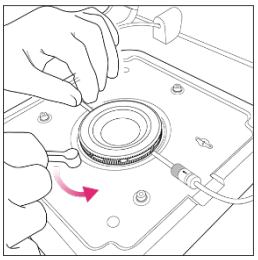
> ***IF*** *the MERSCOPE Instrument will not be used to run a MERFISH experiment for ≥2 weeks, prepare the instrument per the MERSCOPE Instrument Idle Procedure in the MERSCOPE Instrument User Guide* ***IN ADDITION TO (AFTER)*** *the Clean procedure.*
>
> ***IF*** *verification is the only experiment planned for ≥2 weeks, also use MERSCOPE Instrument Idle Procedure in the MERSCOPE Instrument User Guide (as verification runs do not use fluidics).*

- As outlined below, the user interface guides users through cleaning the immersion oil off the high-magnification objective (with lens tissue) after an experiment is complete.
- Leave the filled MERSCOPE Flow Chamber connected to the instrument fluidic lines until the next sample. The MERSCOPE Flow Chamber may be stored in the locked or unlocked position in the stage adapter.
- Leave the MERSCOPE Imaging Cartridge in place until the next sample.
- At the end of cleaning, click **Verification Completed** to return to the home page and start the next experiment.

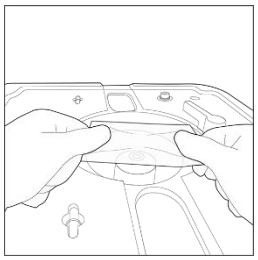
Remove oil from the high-magnification objective lens



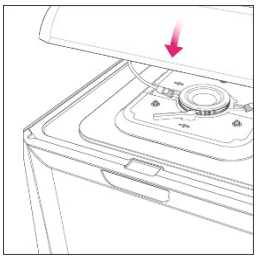
Open the flow chamber lid



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



Clean the high-magnification objective with lens tissue



Return the flow chamber to the stage adapter. Close the flow chamber lid