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Decoding Living Systems

Vizgen MERSCOPE®

at the Earlham Institute

Inside this document

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The Vizgen MERSCOPE® platform

The Vizgen MERSCOPE instrument uses the MERFISH technology, which is based on smFISH probe detection of RNA transcripts. This is an imaging readout as opposed to other platforms which generate sequencing-type data.

Unlike many spatial platforms currently available, the MERSCOPE enables the analysis of all species, and benefiting from high sub-cellular resolution.

With an option of two slide types, they have an imaging area of 1 cm² (small) and 3 cm² (large). Multiple small samples can be placed within this region, allowing for replicates to be analysed on a single slide, optimizing both cost and efficiency.

The Earlham Institute is a **Certified Service Provider** for the MERSCOPE in situ Spatial Transcriptomics Workflow.

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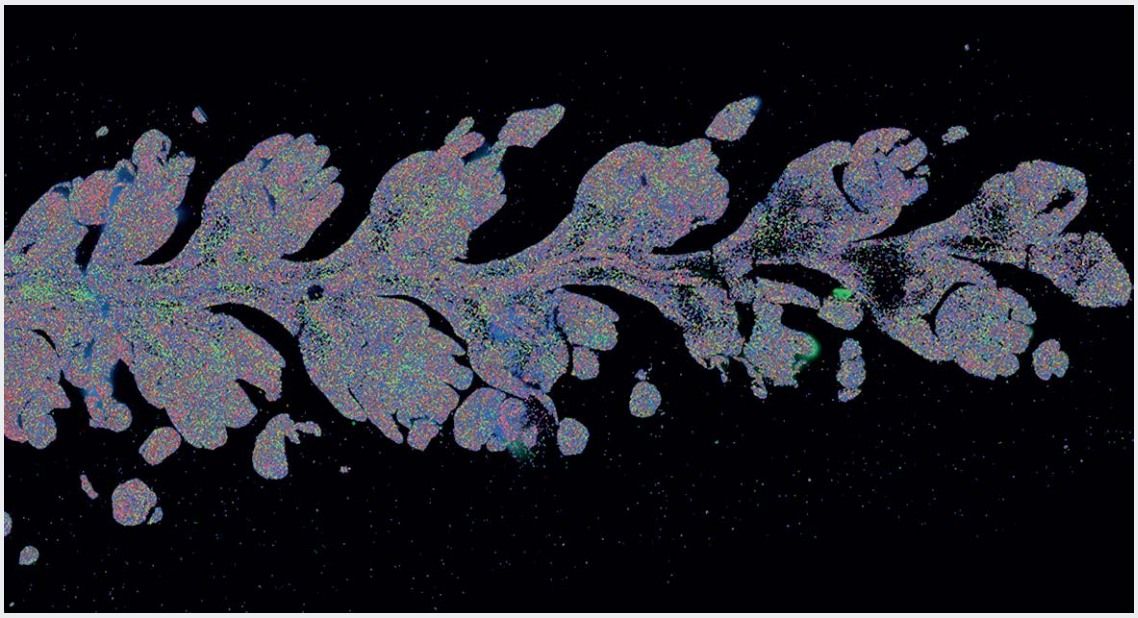

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How does the Merfish technology work?

For each gene, 20-50 probes are used for detection. Each probe has a 25 nt hybridisation sequence, with flanking handles. These handles are made up of sequences that will bind to fluorescent readout probes during sequential rounds of fluidics delivery.

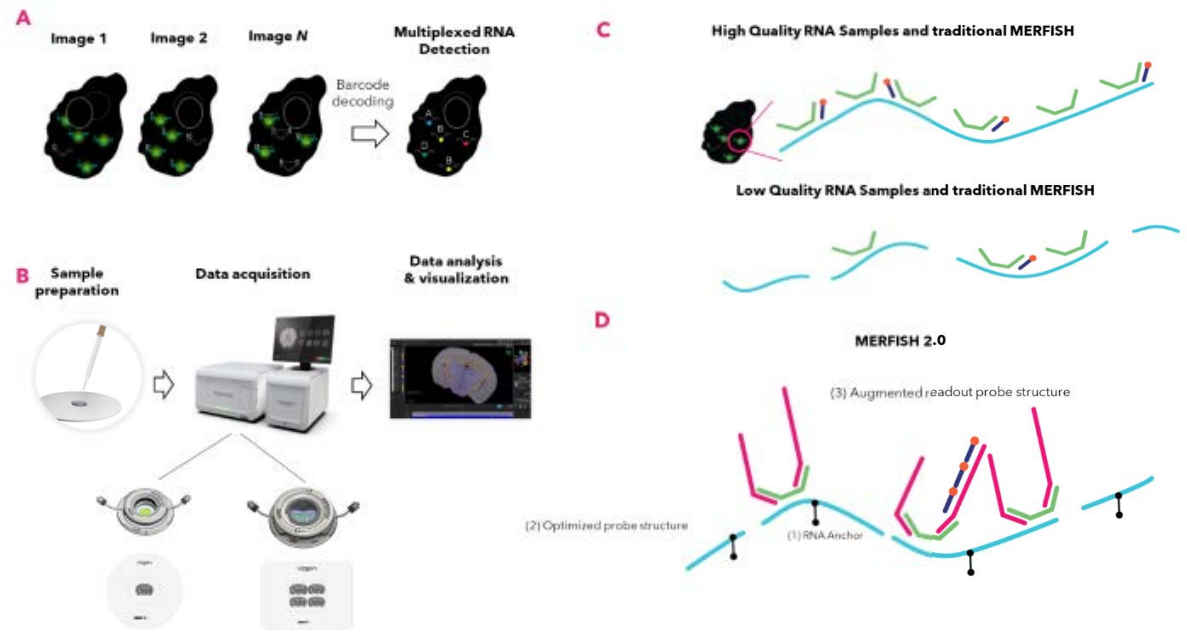
A X60 objective lens images each tile of the imaging area. This is repeated after fluidics deliver the readout probes, and after each laser excitation. Software detects a fluorescent spot at each pixel, during each round of fluids and imaging to piece together a unique molecular barcode, made up of the readout probes assigned to that gene within the panel.

A digital readout made up of spots at each pixel, is overlaid on top of fluorescent images of the stained tissue.

The newly released v2 chemistry, available on both instruments, provides improvements for detection of genes in poorer quality samples (possible RNA degradation) and fluorescent signals are boosted.

> **Please read** our online article for more information:

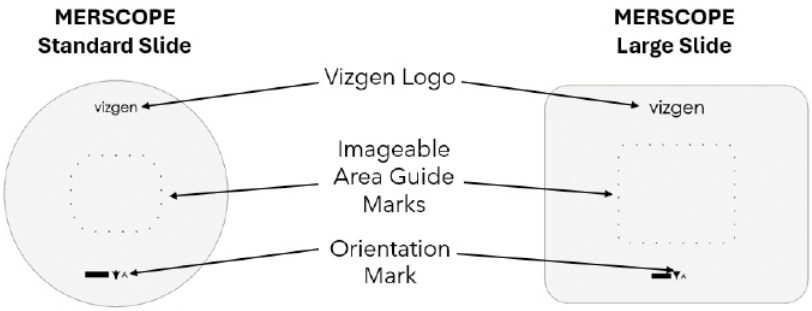
MERFISH 2.0 Chemistry Overview



Credit: Vizgen

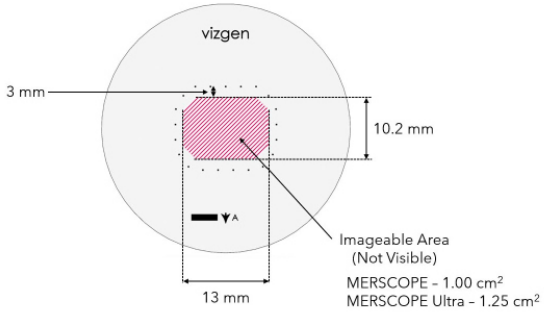
Slide imageable areas

Instrument	Compatible Slides	Maximum Slide Tissue Size Compatibility
MERSCOPE Instrument	MERSCOPE Standard Slide V 2.0	1 cm^2
MERSCOPE Ultra Instrument	MERSCOPE Standard Slide V 2.0	1.25 cm^2
	MERSCOPE Large Slide V 2.0 3	3 cm^2

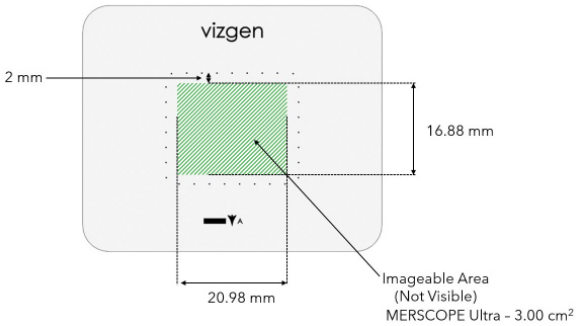


Credit: Vizgen

Histology Guide for Preparing FFPE Samples



MERSCOPE standard slide imageable area and distance from imageable area guides marks.



MERSCOPE large slide imageable area and distance from imageable area guide marks.

Credit: Vizgen

> **Please note:** slides come with a doted area marked. This line is for guidance, and samples should not sit here. There is a margin within this space where the slide stage can reach over the objectives. Samples can occupy beyond the imageable area, but they cannot not be imaged.

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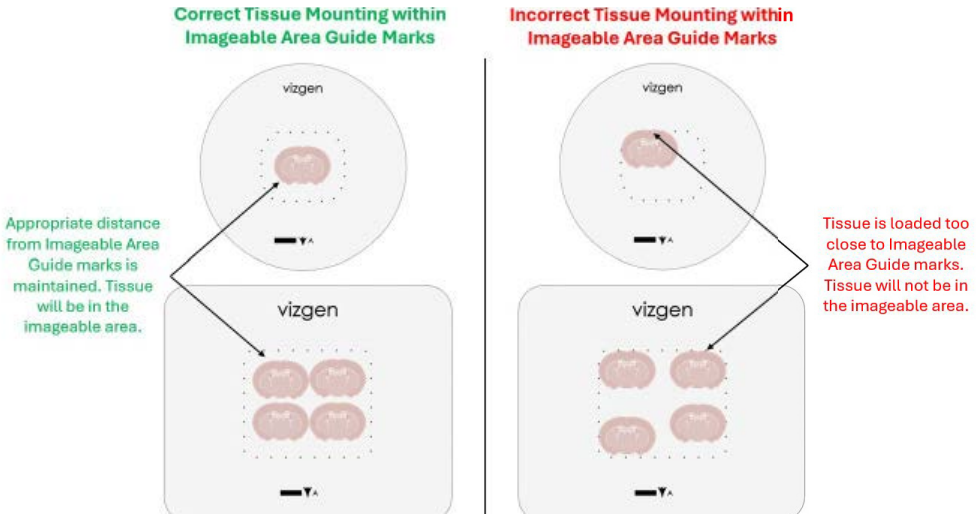
Slide imageable areas

Sample orientation and numbers, requires careful consideration with respect to imageable areas of the various MERSCOPE slides. The diagram below, from the Vizgen histology guide, shows that if samples are small enough, more than one sample can be analysed on the platform, with the most available space on the Ultra large slides.



Credit: Vizgen

Gene panels and protein detection

Gene panels can be different sizes: **140, 300, 500, 960**. A small proportion of the panel numbers (20-40) are set aside as blank probes (controls).

Gene panel design is processed through the Vizgen team. They will be able to utilise data such as RNASeq or scRNASeq, from the same tissue. Generally, a list of gene names with their associated FPKM values as well as a transcriptome or genome reference are needed to order a panel. Expression data should be roughly balanced but important, highly expressing genes can use the auxillary channels. You should be aware that the more of these you have, the less proteins you are able to detect, as they are read using the same channels.

An important consideration is that whereas genes detected in the main gene panel can be counted, genes and proteins detected in the auxillary channel cannot. They will appear as more of a stain, and will differ in intensity. Single cell data is not essential. There are advantages of having single cell data, as this will confirm cell types by including markers and in turn, provide expression data per cell type.

The genes of interest are required to have a unique 200bp region. This means that most differential isoform usage studies are not an option using this technology.

> **Please note:** panels take around **10 weeks** from design submission to delivery from Vizgen.

Vizgen requires the use of a gene panel to detect the genes of interest in your sample type. Probe sets can be tissue and species agnostic. Often, these panels are custom per project.

Customised gene panels come with enough volume for:

- 10 (8) Standard Merscope slides
- 5 (3) Merscope Ultra slides

We suggest that you do not consider your samples number as the maximum slide volume provision but the number in brackets. This number accounts for sample failures which can occur due to adherence, bad RNA integrity, or bad tissue integrity. Any instrument or sample preparation failures will be replaced by Vizgen or Earlham Institute as appropriate.

- Predesigned “small” panels cover 4 standard Ultra or v1 Merscope instrument slides, 2 large Ultra slides.
- Predesigned “large” panels cover 10 standard Ultra or v1 Merscope instrument slides, 5 large Ultra slides.

Please see info here: **https://vizgen.com/predesigned-gene-panels/**

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Gene panels and protein detection

Those pre-designed available are:

- Human Immuno-Oncology
- Human Liver Tumour and Immune profiling
- Human PanCancer Pathways
- Mouse PanNeuro Cell Type

For more information on panels and design please visit the **Vizgen Resources Hub:** vizgen.com/resources

Protein co-detection

Customised protein detection kits are commercially available, with up to 6 proteins and 5 if using the cell boundary staining.

If you are interested in proteins please contact us from more information.

Slides (runs) per reagent

Associated costs are for service provision of the slide preparation, instrument runs, Vizgen kits or consumables and other non-Vizgen consumables. Gene panels, slides, cell boundary stains, protein stains, gene imaging kits and sample prep kits are all purchased directly from Vizgen but costs are included for quotations.

Gene panel comes with enough volume for:

- 10 (8) v1 MERSCOPE slides, or Standard Ultra MERSCOPE slides
- 5 (3) Large Ultra MERSCOPE slides

Protein stain kits enough volume for:

- 20 v1 MERSCOPE slides, or Standard Ultra MERSCOPE slides
- 10 Large Ultra MERSCOPE slides

Samples prep kit come with enough to prepare:

- 10 v1 MERSCOPE slides, or Standard Ultra MERSCOPE slides
- 5 Large Ultra MERSCOPE slides

Slides:

- Standard V2 MERSCOPE slides come in a pack of 20.
- Standard Ultra MERSCOPE slides come in a pack of 10.
- Large Ultra MERSCOPE slides come in a pack of 5.

Gene imaging kits can be purchased as individual units, and one is required per slide (run).

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The stages of processing a new sample

Below is a diagram indicating the major stages of MERSCOPE optimisation for a new sample type.

We **do not** provide sample preparation or sectioning services.

Sample preparation

There are four possible sample preparation options for the MERSCOPE platform;

- Fresh frozen
- Fixed frozen
- Formalin Fixed Paraffin Embedded (FFPE)
- Suspended cells

Sectioning thickness and general guidelines

We **do not** provide sample preparation or sectioning services.

The thickness of your section should be dictated by the diameter of the smallest cells in your tissue. We want to avoid overlapping or stacking of cells.

The MERSCOPE can accept sections of between 3-12um for fresh frozen or fixed frozen samples, 4-5um for FFPE samples. Sections should be well adhered in all regions of your tissue. Any areas that are not well adhered, cannot be focused by the instrument resulting in loss of probe spot detection or blurriness of DAPI staining.

All Vizgen recommendations and guideline documents can be found here:
https://portal.vizgen.com/resources/user-guides

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Sample preparation

Fresh frozen:
Samples are harvested and immediately placed into an embedding mold to be filled with Optimal Cutting Temperature (OCT) resin. Sample blocks are immediately frozen ready for cryosectioning. Sections are taken at a thickness of between 3-12um.

Fixed frozen:
Samples are harvested, dissected and immediately fixed in solutions such as Paraformaldehyde (PFA). Samples will likely require a dehydration stage in a gradient of solutions such as sucrose.

Samples are harvested and immediately placed into an embedding mold to be filled with OCT resin. Sample blocks are immediately frozen ready for cryosectioning. Sections are taken at a thickness of between 7-12um.

Sectioning guidelines for Vizgen samples can be found in MERSCOPE Resource Guide Histology Guide for Preparing Fresh and Fixed Frozen Tissue Samples for Experiments on the MERSCOPE Platform Doc 91600129 Rev B and also MERSCOPE User Guide Fresh and Fixed Frozen Tissue Sample Preparation 91600132 Rev A.

FFPE:
Samples are harvested, dissected and immediately fixed in formaldehyde.

A tissue processor automates the following stages. Dehydration is performed usually in a series of ethanol solutions. Xylene is normally used next in order to Xylene is then used to clear the tissue of fat, and is a solvent in order for the wax to penetrate the tissue.

The tissue is then infiltrated with paraffin wax. Sectioning is performed on a microtome at a thickness of around 5um (3-5um) where sections, or ribbons are transferred to a clean water bath. Optimal sections are selected to adhere to the Merscope slide.

FFPE sectioning guidelines can be found in MERSCOPE User Guide Formalin-Fixed Paraffin-Embedded Tissue Sample Preparation 91600126 Rev B.

It is also possible to perform runs on suspended cells. This is very custom.

Sample optimisation

The tissue you would like to analyse will need to undergo protocol optimisation. This optimisation would normally involve the decision of whether the tissue requires fixation, getting the embedding right and practising sectioning.

Once this is good enough, the next stage we ask is that sections are used as input for RNA extractions. We are looking for good length RNA fragment sizes therefore the RNA will need to be run on a gel-based QC platform for assessment.

Please bear in mind, if you are planning to run tissue sections from multiple tissues types, each tissue type will need to be optimised for both sectioning, as well as the RNA quality. Once these checks are passed, we can proceed to verification.

A big issue with Vizgen MERSCOPE runs is tissue adherence. This needs to be optimised before verification kits are run. You should try this on a Vizgen slide to be sure that it will work on a run. Areas of lifting will cause out of focus regions where staining and probe spots cannot be resolved.

Verification

The verification kits use a single FISH probe to enable cost effective optimisation of tissue clearing, autofluorescence reduction and probe hybridisation so that the very expensive full gene panel runs have the best chance of success. Separate verification kits are also available for protein detection.

For new samples, we will always factor verification kit processing of 5 samples on standard slides, into the timescales and costing. Once we are happy with the results of a verification run, we are able to proceed to full probe runs. This may require 5 verification runs, especially if the sample is challenging.

Pre-made verification kits can be purchased from Vizgen and therefore delivered quickly. They use the Elongation factor 2 (Eef2) gene in mouse and EEF2 in human kits. Custom designed verification kits can be manufactured and purchased from Vizgen for other species (please note that these take up to 4 weeks to be delivered). Selected genes are required to be highly expressed in all cell types as there purpose it to verify the MERFISH protocol is working efficiently throughout the tissue.

Timescales

The instrument schedule can become quite busy, but we will provide an update on the next expected availability during an initial call.

Preparation of the verification slide takes approximately one week, from the sectioned slide stage to running and QC assessment. At this point, we will review our findings with you and provide feedback. This discussion will include our recommendations for further verification tests or confirmation of readiness to proceed with full probe runs.

Full probe runs take one week to prepare for loading. The data processing and transfers take a little longer. We suggest in total this comes to 1-2 weeks. For plant samples this will take 2 weeks minimum. Each slide is prepared and run individually, therefore, please account for this timescale with regard to the number of slides planned. We will aim to contact you as to how well each run performed, within 2 days of the data processing completion.

Data

Raw files constitute a significant portion of the total data generated by the Vizgen MERSCOPE platform. There is ongoing debate about whether retaining these raw files for each project is necessary or if they can be deleted after processing.

To ensure seamless data transfer, we require you to have adequate storage space available to accommodate all project files, including the sizable raw files.

Verification runs amass around 2Tb including raw files. Please allow for storage of 5 of these runs =12Tb.

The MERSCOPE instruments can generate 2-8 terabytes per run (depending on gene panel, slide and tissue size). To transfer such large quantities of data we use **Globus**, which is a secure and reliable transfer process.

Please be aware that if you would like to use our services you will need to be able to receive data via globus, which may require set up with your computing team.

Large data storage at the Earlham Institute will accrue a significant cost over a short amount of time, therefore quick transfer is important.

Data Visualisation software and post-processing

Vizgen use a software called Vizualizer which allows users to explore each area of the tissue as a microscopy image. It has many features such as the selection of the different probes to be visible or not, optimisation of staining etc.

It uses MERSCOPE generated files called VZGs which are mosaiced, stacked, zipped images.

On board image analysis processing performs cell segmentation using an algorithm called Cellpose as default. Raw files can also be retrieved to perform other segmentation algorithms on. This is not a service we offer.

Segmented data are compatible with many downstream single cell analyses such as Seurat or SCAMPI.

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Overview of slide preparation

Stages in v2 chemistry:

(FFPE only) Deparaffinisation and decrosslinking. Paraffin, used as a structural resin in the sectioning process, is dissolved. Decrosslinking reverses the crosslink originally introduced in formalin fixation. Occasionally, this can be performed in fixed frozen tissue too.

Cell boundary staining (mammalian only, also not for neuronal tissue). Cell boundaries are stained using proprietary antibodies against cell membrane proteins. These can be useful (but not imperative) for the analysis stage of cell segmentation (dividing the images up into individual cells).

Optional **protein stains** can be done at this point if required by the user (see section on protein stain kits). These are antibodies against the proteins of interest.

Anchoring pretreatment primes the RNA for anchoring.

Anchoring buffer along with a formamide wash, prepares the RNA for gel embedding.

Gel embedding immobilises the RNA and creates a protective layer around the RNA and the tissue. This prevents tissue lifting during the subsequent clearing (digestive) stage.

Clearing digests away proteins and cellular structures from the background. Only RNA and DNA, and the stains are left in place. This stage is very sample specific, and therefore can take between 1-7 days.

Autofluorescence quenching/photobleaching is where the sample is overexposed to UV light to dull natural emission properties. This works well with low levels of autofluorescence such as in much mammalian tissue or young plant material, but generally, if the emission is high, then the photobleacher will be an ineffective solution for the issue.

Encoding Probe hybridisation is where the probe set is applied to the tissue, finding the unique complementary RNA sequences within the in situ location, now suspended in the gel matrix.

Enhancer probe hybridisation is where the v2 chemistry is introduced. These probes create an extension to the encoding probe handles, which amplify the fluorescent signal. This improves the signal and therefore the detection of each probe without the bias of PCR duplication used in other platform providers.

Staining DAPI is used to stain DNA in the nuclei. PolyT is used to stain the gene regions such as the cytoplasm. This is performed prior to an instrument run.

Overview of slide preparation

Each **instrument run requires** a gene imaging cartridge which supplies the MERSCOPE with all fluids required for reading out the probe locations.

A run is set up, the slide is situated into an adapter, over the top of the microscope objective and is set up so that the software is aware of which probes it and how many it is expecting (using a “codebook csv file”). The MERSCOPE will quickly take a X10 image of the whole imageable area. From this, we can then define the region of interest (s) which can be made up of either 10 regions or the maximum area of the run type (see above for detailed info).

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