

91600100 Rev C

MERSCOPE[®] Vizualizer

User Guide

Desktop Version



NOTICES

| | |
|-------------------|-------------|
| Document number | 91600100 |
| Document revision | Rev C |
| Revision date | August 2023 |

Legal notices

ALL PRODUCT(S) AND SERVICES DESCRIBED HEREIN ARE INTENDED FOR RESEARCH USE ONLY, AND NOT FOR USE IN DIAGNOSTIC OR TREATMENT PROCEDURES OR FOR ANY OTHER USE.

FAILURE TO COMPLETELY READ AND EXPLICITLY FOLLOW ALL OF THE INSTRUCTIONS CONTAINED HEREIN MAY RESULT IN DAMAGE TO THE PRODUCT(S), INJURY TO PERSONS, INCLUDING TO USERS OR OTHERS, AND DAMAGE TO OTHER PROPERTY. VIZGEN, INC. ("Vizgen") DOES NOT ASSUME ANY LIABILITY ARISING OUT OF THE IMPROPER USE OF THE PRODUCT(S) DESCRIBED HEREIN (INCLUDING PARTS THEREOF OR SOFTWARE) OR ANY USE OF SUCH PRODUCT(S) OUTSIDE THE SCOPE OF THE EXPRESS WRITTEN LICENSES OR PERMISSIONS GRANTED BY VIZGEN.

Nothing contained herein shall constitute any warranty, express or implied, as to the performance of any product(s) described herein. Any and all warranties applicable to any product(s) and software are set forth in the applicable terms and conditions of sale accompanying the purchase of such product(s). Vizgen provides no warranty and hereby disclaims any and all warranties as to the use of any third-party product(s) or protocols described herein. The use of product(s) described herein is subject to certain restrictions as set forth in the applicable terms and conditions of sale accompanying the purchase of such product(s).

Updates to product(s) and software may be required to enable customers to use product(s). Failure to update the product(s) or software when provided by Vizgen immediately terminates any warranty thereon. In the event of a product failure resulting from an update, such failed product will be subject to the warranty set forth in the terms and conditions agreed to in connection with the purchase of such product, if any, only if such product is covered by such warranty at the time of such failure. Vizgen is not required to repair, replace, or take any other remedial action with respect to any product not covered under such warranty.

Vizgen® and MERSCOPE® are trademarks of Vizgen. All rights in the trademarks, service marks, trade dress, logos and copyrights are owned by Vizgen, Inc. and fully reserved. All other brands and marks contained herein are the property of their respective owners.

This document and its contents are proprietary to Vizgen, Inc. and its affiliates, and are intended solely for the contractual use of its customer in connection with the use of the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose and/or otherwise communicated, disclosed, or reproduced in any way without the prior written consent of Vizgen. Vizgen does not convey any license under any patent, trademark, copyright, or common-law rights, nor similar rights of any third parties. The instructions in this document must be strictly and explicitly followed by qualified and properly trained personnel in order to ensure the proper and safe use of the product(s) described herein. All of the contents of this document must be fully read and understood prior to using such product(s).

© 2023 Vizgen, Inc. All rights reserved.

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/>

VERSION CHANGE NOTES

Summary of substantive changes from Rev B to Rev C

| Brief description of change | Page(s) |
|--|--------------|
| Mac operating system recommendations | 14 |
| Assemble experiment multiple dataset selection | 15 |
| Menu bar File>Import data options flow/organization | 12-13, 16-18 |
| Menu bar File>Export data options flow/organization | 11, 18-20 |
| Rename/deselect 2D arrays for scatterplot in categories dialog box | 17-18 |
| Import/export in PARQUET format | 16-17, 19 |
| Auto-save feature | 20 |
| Imaging channels up to 16 | 21, 27 |
| Menu bar Edit>Save custom cells/ROI groups as new copy | 21 |
| Menu bar Help>Archive Logs | 23 |
| Tooltips | 24 |
| Transcript size slider | 27 |
| Layer/z-plane specifics | 27 |
| 'Group by category' updated to 'Group by active category' | Throughout |
| Create a create and import a cell group | 33-34 |
| Color scheme slider appearance/operation | 36, 40 |
| Scatterplot active feature set indicator when features colored by expression | 41 |
| Move to/create a new gene group from gene settings | 44 |
| Create a new gene group from highlighted genes | 45 |
| Heatmap navigation, zoom/scale, export image icons and organization | 48 |
| Export filtered expression matrix from heatmap | 48 |
| Heatmap toolbar - reorganization of tools from heatmap settings | 49-50 |
| Hierarchical clusters slider moved from heatmap toolbar to heatmap axes | 49 |
| Recalculate hierarchical clustering button | 49 |
| Update - heatmap scale direction (x, y, both) option removed | - |
| | |
| Details panel updates (general) | |
| Data reorganization | - |
| Gene list of selected feature added | - |

TABLE OF CONTENTS

| | |
|---|-----------|
| INTRODUCTION..... | 7 |
| MERFISH Technology | 7 |
| The Vizgen MERSCOPE Platform Solution | 7 |
| The MERSCOPE Workflow | 8 |
| Broad Application..... | 8 |
| VIZUALIZER SOFTWARE USER INTERFACE OVERVIEW | 9 |
| QUICK START ORIENTATION..... | 10 |
| Installation | 10 |
| Create a First Experiment..... | 10 |
| Generate and Import Custom Cell Type Annotations | 11 |
| Open Custom Cell Clustering Analysis | 11 |
| SYSTEM RECOMMENDATIONS..... | 14 |
| MENU BAR..... | 15 |
| Software Versions | 15 |
| File > Assemble Experiment | 15 |
| File > Import Data | 16 |
| File > Import Data > Metadata | 16 |
| File > Import Data > Geometry > [Name of ROI Feature Set] | 18 |
| File > Export Data | 19 |
| File > Export Data > Metadata..... | 19 |
| File > Export Data > Geometry > [Name of ROI Feature Set] | 20 |
| Import/Export Leiden Colors From/To Scanpy..... | 20 |
| File > Open Experiment | 20 |
| File > Save Experiment..... | 20 |
| Edit > Manage Image Channels..... | 21 |
| Edit > Manage ROI Sets | 21 |
| Edit > Save Custom [Feature Set] Groups as New Copy | 21 |
| View..... | 22 |
| Help..... | 23 |
| Help > Archive Logs..... | 23 |
| SPATIAL VIEW WINDOW..... | 24 |
| Tooltips | 24 |
| Navigation and Views..... | 24 |
| Zoom and Scale..... | 25 |
| Information Panel | 25 |
| Export Image | 26 |
| Spatial Settings..... | 26 |
| Additional Settings (Under Spatial Settings Menu) | 27 |
| DETAILS AND SELECTED ITEMS PANELS..... | 28 |
| Details Panel..... | 28 |

| | |
|---|-----------|
| Selected Items Panel - Cells | 28 |
| Selected Items Panel - ROI | 29 |
| SPATIAL FEATURES PANEL - CELLS..... | 30 |
| Select the Cells Feature Set | 30 |
| Cells..... | 30 |
| Active Category | 31 |
| Selection Toolbar (Cells) | 31 |
| Cell Groups..... | 33 |
| Checked Cell Groups | 35 |
| Unchecked Cell Groups..... | 35 |
| Cell Group Settings | 35 |
| Cell Color Options..... | 36 |
| SPATIAL FEATURES PANEL - REGIONS OF INTEREST | 37 |
| Select an ROI Feature Set..... | 37 |
| Selection Toolbar (ROIs) | 37 |
| ROI Construction and ROI Groups | 38 |
| Checked ROI Groups..... | 39 |
| Unchecked ROI Groups..... | 39 |
| ROI Group Settings | 39 |
| ROI Color Options..... | 40 |
| SCATTERPLOT | 41 |
| Translate and Zoom | 41 |
| Select Array | 41 |
| Export Image | 41 |
| Selection Toolbar (Cells or ROI)..... | 42 |
| GENES TREE | 43 |
| Checked Genes | 43 |
| Unchecked Genes | 43 |
| Gene Color Settings..... | 44 |
| Gene Settings..... | 44 |
| Gene Highlighting | 45 |
| Gene Groups | 46 |
| Search for Genes | 47 |
| Gene Group Settings..... | 47 |
| HEATMAP | 48 |
| Navigation and Selection | 48 |
| Zoom and Scale | 48 |
| Export | 48 |
| Heatmap Toolbar..... | 49 |
| Heatmap Settings..... | 50 |
| Heatmap Highlighting | 51 |

INTRODUCTION

MERFISH Technology

Multiplexed error-robust fluorescence in situ hybridization (MERFISH) is a spatially resolved single-cell transcriptome profiling technology. MERFISH combines the power of single-cell transcriptomics with spatial biology by directly visualizing and counting RNA transcripts from 100s to >10,000 genes in cells or tissue sections.

MERFISH advances the power of single-molecule fluorescence in situ hybridization (smFISH) – the gold standard of RNA quantification – with error robust barcoding, combinatorial labeling, and sequential imaging to greatly expand the multiplexing capacity. MERFISH enables researchers to map the molecular, cellular, and functional composition of biological systems with spatial context.

The Vizgen MERSCOPE Platform Solution

The Vizgen MERSCOPE Platform Solution is comprised of the Vizgen Gene Panel Design Portal, MERSCOPE reagent kits, the MERSCOPE Instrument, the MERSCOPE Analysis Computer, and the MERSCOPE Vizualizer software to streamline the acquisition of high quality MERFISH data and data interpretation.

Reagent kits facilitate sample preparation. The automated MERSCOPE Instrument integrates high-resolution imaging and fluidics to automatically acquire a full MERFISH dataset. The MERSCOPE Vizualizer software automates image processing and offers interactive visualization tools to explore MERFISH data.



The MERSCOPE Workflow

The MERSCOPE Workflow begins with either designing a MERSCOPE Custom Gene Panel or ordering a MERSCOPE Predesigned Gene Panel.

Design a custom gene panel. The intuitive Vizgen Gene Panel Design Portal makes recommendations on your custom gene panel for optimal performance in a MERFISH measurement. For example, the portal highlights genes that might cause optical crowding or cannot be accurately targeted. Vizgen is currently offering custom gene panels for up to 140 genes, up to 300 genes, and up to 500 genes.

Order your MERSCOPE Gene Panel. MERSCOPE Custom Gene Panels and MERSCOPE Predesigned Gene Panels are delivered in a ready-to-use format. Once the gene panel is finalized, users will be able to download their panel-specific MERSCOPE Codebook to the MERSCOPE Instrument.

Prepare your sample. The Vizgen sample preparation user guides take users through each step of the MERSCOPE sample preparation process.

Load and run the MERSCOPE Instrument. The MERSCOPE Slide is assembled into the MERSCOPE Flow Chamber and then loaded into the instrument along with a MERSCOPE Imaging Cartridge. Users define regions of interest on the MERSCOPE Slide and initiate the fully automated instrument run.

Data Processing and Visualization. The MERSCOPE Instrument Software (in combination with the MERSCOPE 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 (HDF5 [MERSCOPE Instrument Software v231 or earlier] or PARQUET [v232 or later]), and a binary for use with the MERSCOPE Vizualizer software. The MERSCOPE Platform Solution 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.

Broad Application

The MERSCOPE Platform Solution has broad application in both fundamental biology and medicine – from basic science, to drug discovery, to clinical pathology. Find out more at <https://vizgen.com/applications/>.



Oncology



Immunology



Neuroscience



Infectious Disease

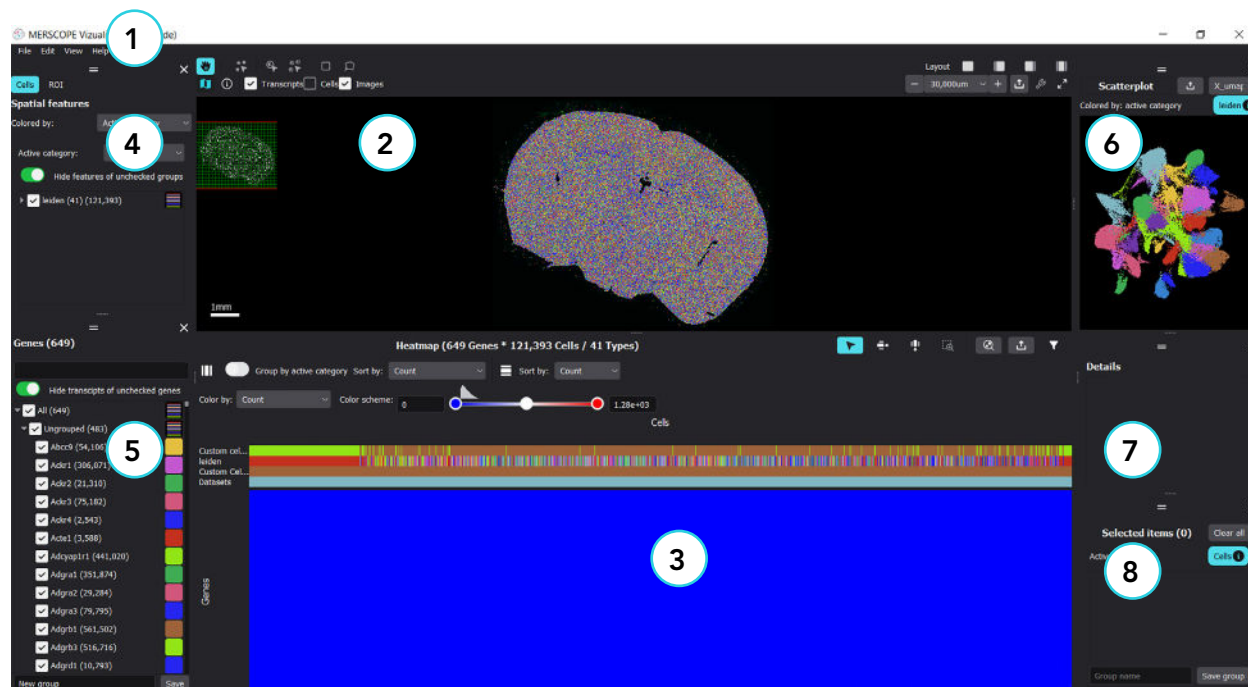


Developmental Biology &
Regenerative Medicine

VIZUALIZER SOFTWARE USER INTERFACE OVERVIEW

| | | |
|-----------------------|--|--|
| 1 Main menu bar | File | Open experiment, recent, import data, export data, save experiment, assemble experiment |
| | Edit | Manage image channels and ROI sets, save a copy of custom feature set groups |
| | View | Control the visibility of the floating panels surrounding the spatial view window |
| | Help | Version information, about Qt (the C++ framework used for application development), open the report folder, archive logs |
| 2 Spatial view window | Tissue visualization, view the MERSCOPE Instrument output data | |
| 3 Heatmap | A two-dimensional view of cells by gene count data | |
| 4 Spatial features | Manage the active category, cell groups, and ROIs. Each feature set (cells, ROI sets) is addressed individually | |
| 5 Genes | View, select, and create gene groups | |
| 6 Scatterplot | Display two-dimensional arrays, such as cells in dimensionality reduced gene expression space (e.g., UMAP/tSNE*) or other user-imported two-dimensional arrays (e.g., cell volume versus transcript count) | |
| 7 Details | Information on a selected gene, cell, or ROI | |
| 8 Selected items | A list of selected cells and ROIs, and create cell and ROI groups | |

*UMAP, uniform manifold approximation and projection; tSNE, t-distributed stochastic neighbor embedding.



QUICK START ORIENTATION

The MERSCOPE Vizualizer software enables interactive visualization and exploration of all components of a MERFISH measurement acquired on the MERSCOPE Instrument, including the detected transcripts and the segmented cell boundaries overlaid on the underlying images. The MERSCOPE Vizualizer uses data in the .VZG format, which is a compressed representation of the MERSCOPE Instrument output data optimized for efficient visualization.

- *The quick start orientation narrative is intended to help users quickly understand basic functions and get started with the MERSCOPE Vizualizer software. Links to more detailed descriptions of software operations and additional features are included in the narrative.*

Installation

- ➔ Install the MERSCOPE Vizualizer software using the installer executable and follow the installation prompts.
- ➔ Installation requires registering your installation with Vizgen. To register, follow the prompts, insert an email address, and enter the activation code that is shown on the registration landing page and emailed to you as a copy. Vizgen will use the provided email address to send notifications about new MERSCOPE Vizualizer software releases. Check [Software Versions](#) for any potential incompatibility between experiments assembled in different versions.
- *DO NOT install the MERSCOPE Vizualizer software on the MERSCOPE Instrument or the MERSCOPE Analysis Computer.*

Create a First Experiment

- ➔ An experiment in the MERSCOPE Vizualizer software is a collection of datasets, where each dataset is a region of interest (ROI) defined on the MERSCOPE Instrument software during data acquisition. An experiment can contain one or more datasets that use the same MERSCOPE Gene Panel.
- ➔ A dataset **.VZG file** can be found in the merfish_output directory on the Z: drive of the MERSCOPE Instrument. Copy the .VZG file to your computer either over the network or via a USB drive.
- ➔ Open the MERSCOPE Vizualizer software and assemble the experiment by selecting **File > Assemble experiment** from the [MENU BAR](#).
- ➔ Enter a name for the new experiment in the **Experiment name** text field. Click the folder next to **Select datasets** to open a file browser and **select the .VZG file(s)** copied from the MERSCOPE Instrument. Select an output location and click **Build** to assemble the experiment.

- ➡ The MERSCOPE Vizualizer software will unpack the .VZG file(s) into the specified experiment directory. Once the experiment is created, the user interface will populate with the experiment data.
- ➡ Initially, the center z-plane of transcripts and images are displayed in the [SPATIAL VIEW WINDOW](#) (refer to [Spatial Settings](#) for more information on z-planes). All transcripts can be toggled on and off in the [Spatial Settings](#) menu (the tools icon above the top right of the **spatial view window**) and selecting **All** in the **Z-plane** menu for the **transcripts** layers. Cells and images can also be toggled on and off in this menu.
- ➡ To quickly visualize the measured expression of each gene, click on the name of a gene in the [GENES TREE](#) in the **genes** panel and use the up/down arrow keys to scroll through the list. When a gene is highlighted in the genes list, only the highlighted gene is displayed in color (the others are displayed gray) in the **spatial view window** (the **transcripts** layer must be checked). Press **Esc** to reset the selection.
- *Note that ROIs on the MERSCOPE Instrument are **different** from ROIs defined in the MERSCOPE Vizualizer software in the [SPATIAL FEATURES PANEL - REGIONS OF INTEREST](#).*

Generate and Import Custom Cell Type Annotations

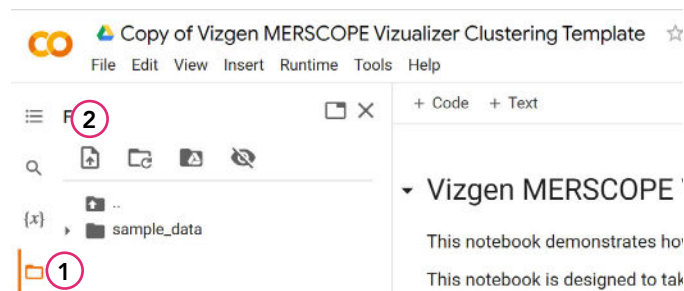
- ➡ The MERSCOPE Vizualizer software is able to visualize custom cell type annotations. To generate custom cell type annotations and populate the [SCATTERPLOT](#) with a UMAP representation of the data, the cell by gene matrix can be exported from the MERSCOPE Vizualizer software, loaded into custom analysis tools, exported with cell type annotations, and imported back into the MERSCOPE Vizualizer software with cell type annotations. MERSCOPE Vizualizer exports and imports cell annotations in the AnnData (HDF5), CSV, or PARQUET formats.
- ➡ To export an AnnData (.HDF5) file, select **File > Export data > Metadata > [feature set]** from the [MENU BAR](#). Select AnnData data format, navigate to the save location, and click **Export**.

Open Custom Cell Clustering Analysis

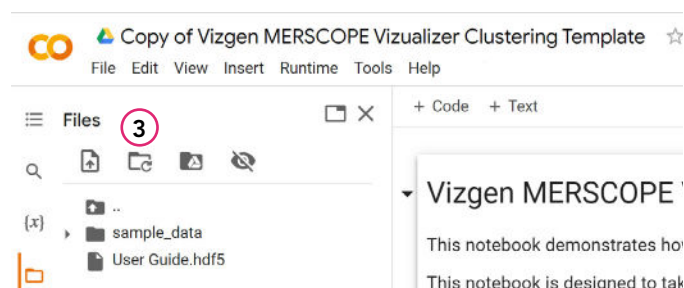
- *To execute this process, uploaded files must **NOT** have a **_clustered** suffix to the .HDF5 file name (an error will occur during Load AnnData). If a file has previously been processed (clustered) and the file name changed to remove **_clustered**, errors may occur during processing.*
- ➡ A Vizgen template Jupyter Notebook is hosted on Google CoLab. To cluster the cells in the exported AnnData file in the Google CoLab notebook, navigate to <https://vizgen.com/merscope-clustering/> and click **ACCESS THIS RESOURCE**.
- *Google CoLab is a free service with a RAM limitation of approximately 13 GB, which may*

restrict the processing of larger datasets containing over 100,000 cells and 500 genes

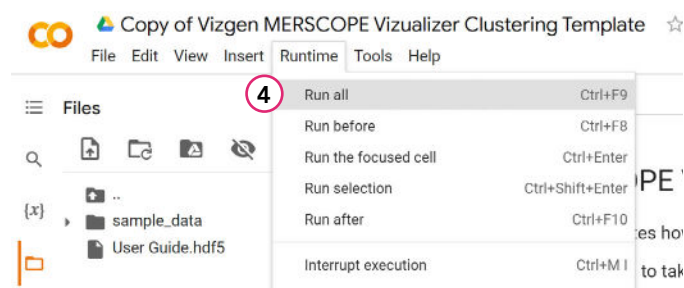
- ➔ Make a copy of the notebook in your account by selecting **File > Save a copy in Drive**.
- ➔ Click on the folder icon (1) to the left of the browser window to open the **Files** tab.
- ➔ When the **Files** tab is open, click on **Upload to session storage** (2) to upload a .HDF5 file.



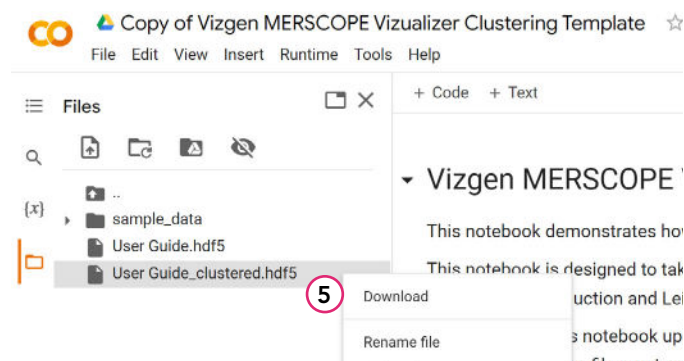
- ➔ Click **Refresh** (3) to ensure the file is available and shown in the list.



- ➔ After the file has uploaded (note the progress wheel at the bottom of the **Files** tab), select **Runtime > Run all** (4) to execute all cells in the notebook.



- ➡ The notebook will run clustering analysis and save the clustered file with a **_clustered** suffix to the file name (it may take some time to appear). Right-click on the file name to download (5) the clustered AnnData file from the CoLab instance.



- ➡ Import the clustered AnnData file into the MERSCOPE Vizualizer by selecting **File > Import data > Metadata > [feature set]** from the [MENU BAR](#). Select AnnData data format, navigate to the AnnData file, and click **Import**.
- ➡ Both the clustering results and the two-dimensional representation of gene expression (e.g., UMAP) can be imported.
 - *There are many parameters that can be adjusted to obtain the most biologically relevant cell clustering parameters for a given dataset. For a new dataset, the cell filtering criteria and clustering algorithm parameters should be carefully considered.*
- ➡ After the clustering annotations and UMAP are imported, the [SCATTERPLOT](#) will display the imported UMAP and a new category corresponding to the new clustering annotations is available and selectable from the [SPATIAL FEATURES PANEL - CELLS](#). To color the cells using the clustering annotations, select the clustering annotations in the **Active category** menu, select **Active category** in the **Color by** menu, and ensure the **cells** layer is toggled on in the [Spatial Settings](#) menu.
- ➡ The gene expression for each cluster can be displayed in the [HEATMAP](#). Toggle on the **Group by active category** slider in the [Heatmap Toolbar](#).
- ➡ The UMAP can be colored by gene expression by selecting **Count** or **Z-score** (among others) from the **Color by** menu in the [SPATIAL FEATURES PANEL - CELLS](#).

SYSTEM RECOMMENDATIONS

For optimal performance, the MERSCOPE Vizualizer software should be run on a system with the following minimum criteria:

- Processor: Intel Core i9
- RAM: 32 GB
- Graphics: NVIDIA GeForce RTX 3080-10 Gb
- SSD Capacity: 1 TB

The MERSCOPE Vizualizer software can be run on a system with lower capability, but may result in suboptimal performance, especially for large datasets.

macOS 12 (Monterey) is recommended, where applicable.

In the absence of an optimal graphics card, the MERSCOPE Vizualizer software can be run on a GPU-enabled AWS EC2 instance. Install NVIDIA drivers following the instructions under Option 4: NVIDIA gaming drivers (G5 and G4dn instances) at the following link:

- <https://docs.aws.amazon.com/AWSEC2/latest/WindowsGuide/install-nvidia-driver.html#nvidia-installation-options>

MENU BAR

Software Versions

After an experiment is assembled in version 2.2 or 2.3, it cannot be opened in version 2.1 because the folder structure of the assembled experiments are different. The MERSCOPE Vizualizer software version is found under the [Help](#) menu.

File > Assemble Experiment

To assemble an experiment:

- ➔ **File > Assemble experiment.**
- ➔ Enter **Experiment name** (this is then shown in the top bar of the MERSCOPE Vizualizer software window).
- ➔ Click the **Select output folder** icon to save MERSCOPE Vizualizer metadata.
- ➔ Click the **Select datasets** folder icon to select datasets (.VZG files).
- ➔ Click **Build**.
- *Multiple datasets can be assembled, but they must be from samples measured with the same MERSCOPE Gene Panel. To assemble multiple datasets, repeatedly click **Select datasets** and select the datasets for inclusion in the experiment. If datasets are in the same folder, use CTRL-SHIFT-select to simultaneously select multiple datasets.*
- *Assembling an experiment may take some time. A progress bar across the top of the **spatial view window** shows progress.*
- *The MERSCOPE Vizualizer software creates a new folder with the **experiment name** in the **output folder**.*
- *Only the **transcripts** and **images** layers are visible by default in the **spatial view window** when an experiment is loaded. Refer to [Spatial Settings](#) for more information.*

File > Import Data

The MERSCOPE Vizualizer software supports the import of data to feature sets via:

- The open HDF5 format: <https://www.hdfgroup.org/solutions/hdf5/> (annotated data, AnnData).
- .CSV files or .PARQUET files (categories files and numeric categories files). A numeric categories file imports data to the **scatterplot**. Data in .CSV and .PARQUET files must be organized in the same way as exports from the MERSCOPE Vizualizer software.
- .CSV files of ROI geometry data (of **ROI feature sets** only - this option is not available for the **cells feature set**).

File > Import Data > Metadata

After processing and analyzing annotated data, it can be imported into the MERSCOPE Vizualizer software with additional information (e.g., clusterization).

Each experiment contains two default cell categories:

- **Datasets** is an immutable cell category. This category contains all the cells in each dataset.
- **Custom Cell Groups**. When an experiment is first assembled, a single cell group exists in this category (unclustered), which contains all the cells in a dataset. Custom cell groups can be created within this category.

Each experiment contains one default ROI category:

- **Custom ROI Groups**. When an experiment is first assembled, a single ROI group exists. Custom ROI groups can be created within this category.

In addition to default cell/ROI categories, additional cell /ROI categories can be imported with annotated data (e.g., Leiden). Cell/ROI categories can be linked (nested) during import.

In an experiment assembled from multiple datasets (.VZG files), an exported metadata file contains all cells/ROIs from all datasets in the experiments, combined. In an imported metadata file, the clustering applies to all cells/ROIs in the experiment.

To import annotated data (default):

- *Advanced import options are on the following page. If annotated data has previously been imported, the advanced **Categories** menu automatically appears.*
- ➡ **File > Import data > Metadata > select the spatial feature set** (cells or an ROI) to import data into.

- ➔ Select the data format (AnnData [.HDF5], .CSV, or .PARQUET), navigate to the file location, and click **Import**. A **Categories** dialog box appears.
- ➔ Check the categories for import.
- ➔ Data available for the **scatterplot** (e.g., UMAP, tSNE) are imported by default, and can be renamed in the dialog box (under two-dimensional arrays for scatterplot). If not required, uncheck the appropriate option. Multiple selections can be checked/unchecked and a menu in the **scatterplot** window controls the data being displayed.
- ➔ Click **Import**.
- *The imported cell categories appear in the **active category** selection menu in the **cells feature set** in the **spatial features** panel and the **scatterplot** is populated.*
- *If a **spatial feature set** is empty (no features) an error message appears.*

To import annotated data (advanced):

- ➔ **File > Import data > Metadata >** select the **spatial feature set** (cells or ROI) to import data into.
- ➔ Select the data format of the file (AnnData [.HDF5], .CSV, or .PARQUET), navigate to the file location, and click **Import**. A **Categories** dialog box appears.
- ➔ Click the **Show advanced settings** button (if available - if annotated data has previously been imported, the advanced **Categories** menu automatically appears).
- ➔ Check the categories for import.
- ➔ Check a cell/ROI category in the **Existing experiment metadata** list to import annotated data for that category.
- *The default cell/ROI categories (datasets and custom cell/ROI groups) are already selected in the existing experiment metadata list.*
- ➔ Cell/ROI categories can be linked. Select a subcategory to nest it under a category.
- *Linking imported cell/ROI categories may be useful when there is a connection between them - e.g., coarse and fine clusters.*
- ➔ Cell/ROI categories can be renamed. Click on the name and enter the new name. Each category should have a unique name.
- *If a previously used name is entered, the MERSCOPE Vizualizer software automatically renames the category to be imported to give it a unique name.*

- *Default cell categories (datasets and custom cell groups) cannot be renamed or linked. Default ROI categories can be removed only if the entire ROI feature set is removed.*
- ➔ Data available for the **scatterplot** (e.g., UMAP, tSNE) are imported by default, and can be renamed in the dialog box (under two-dimensional arrays for scatterplot). If not required, uncheck the appropriate option. Multiple selections can be checked/unchecked and a menu in the **scatterplot** window controls the data being displayed.
- ➔ Click **Import**.
- *The imported cell/ROI categories appear in the **active category** menu in the **feature set's spatial features** panel and the **scatterplot** is populated (as applicable).*
- *If a ROI feature set is empty (no features) an error message appears.*

File > Import Data > Geometry > [Name of ROI Feature Set]

To import the coordinates of ROI polygon vertices (coordinates should be in well-known text [WKT] format):

- ➔ **File > Import data > Geometry > [Name of ROI feature set].**
- ➔ Navigate to the .CSV files for import.
- ➔ Click **Open**.
- *The ROI groups will appear in the **spatial features** panel and the **spatial view** window.*

File > Export Data

The MERSCOPE Vizualizer software supports the export of data from feature sets to:

- The open HDF5 format: <https://www.hdfgroup.org/solutions/hdf5/> (annotated data, AnnData).
- .CSV files or .PARQUET files (expression matrix files, categories files, and numeric categories files for **cells and ROI feature sets**; and feature metadata files for **ROI feature sets**). The feature metadata files contain feature volume/area and coordinates.
- .CSV files of ROI geometries (to **ROI feature sets** only - this option is not available for the cells feature set).

File > Export Data > Metadata

Exported annotated data can be processed and analyzed in third-party tools for analyzing single-cell gene expression data (e.g., Scanpy <https://scanpy.readthedocs.io/en/latest/#>).

In an experiment assembled from multiple datasets (.VZG files), an exported metadata file contains all cells from all datasets in the experiments, combined.

To export annotated data:

- ➔ **File > Export data > Metadata >** Select the spatial feature set (cells or ROI) to export data from.
- ➔ Select the format of the file (AnnData [.HDF5], CSV, or PARQUET).
- ➔ For export to .CSV and .PARQUET files: Check the files for export (expression matrix file, categories file, numeric categories file, and/or feature metadata file) and navigate to the location to save the files. Select the data type for the expression matrix file (count, z-score, normalized count, normalized z-score) from the menu.
- ➔ Confirm all save locations and file names are per preferences.
- ➔ Click **Export**.

File > Export Data > Geometry > [Name of ROI Feature Set]

To export the coordinates of ROI polygon vertices:

- ➔ File > Export data > Geometry > [Name of ROI feature set].
- ➔ Navigate to the location to save the .CSV files.
- ➔ Click **Save**.

Import/Export Leiden Colors From/To Scanpy

If a color scheme is applied in Scanpy (e.g., Leiden), the color scheme will be imported with annotated data file and displayed in the MERSCOPE Vizualizer software. Any changes made to the colors in the MERSCOPE Vizualizer software will be exported in the AnnData file and may be imported into Scanpy.

File > Open Experiment

To open an experiment:

- ➔ File > Open experiment.
- ➔ Select the folder with the experiment data.

It is also possible to open a recent experiment via **Recent > [name of experiment]**.

- *The MERSCOPE Vizualizer software opens the data folder and displays the experiment. If the experiment has annotated data, the software also loads all annotations.*

File > Save Experiment

Assembled experiments are auto-saved after any changes are made in the application. All imported data and data created in the MERSCOPE Vizualizer software (custom cell groups, ROIs, gene groups, custom colors of cell/ROI/gene groups) are saved.

To manually save an experiment at any time:

- ➔ File > Save experiment.
- *All imported data and data created in the MERSCOPE Vizualizer software are automatically saved on closing the application.*

Edit > Manage Image Channels

Manage the image channels available in the experiment by unchecking/checking the boxes next to each channel. DAPI and PolyT channels are included in all experiments. Other imaging channels (up to 16) are available for import based on the use of auxiliary cell boundary stains/ other auxiliary stains during data acquisition. Selected image channels are listed in the [Spatial Settings](#) menu.

Edit > Manage ROI Sets

ROIs can be defined. There is one empty ROI feature set by default (named ROI). ROIs can be grouped in each ROI feature set.

To manage ROI feature sets:

- ➡ Edit > Manage ROI sets.
- ➡ Click **Add feature set** to add a new ROI set.
 - A new ROI set with a default name appears in the dialog box. The default name can be changed in the dialog box. The name of the default ROI set (ROI) can also be changed from the dialog box. The ROI set name should be unique.
- ➡ Rename the ROI set or accept the default name and click **OK**.
 - Multiple ROI sets can be added from the same dialog box.
 - A new ROI set appears in the **spatial features** panel.
 - ROI sets can be deleted or renamed from the **manage ROI sets** dialog box.

Edit > Save Custom [Feature Set] Groups as New Copy

To save custom cells/ROI groups as a new copy:

- ➡ Edit > Save Custom Cells groups as new copy
- ➡ Insert a name for the copy and click **OK**.
 - The copied and renamed custom cells/ROI groups can be selected in the **spatial features panel** from the [Active Category](#) menu. The copied group is immutable (only group colors can be changed by the user).

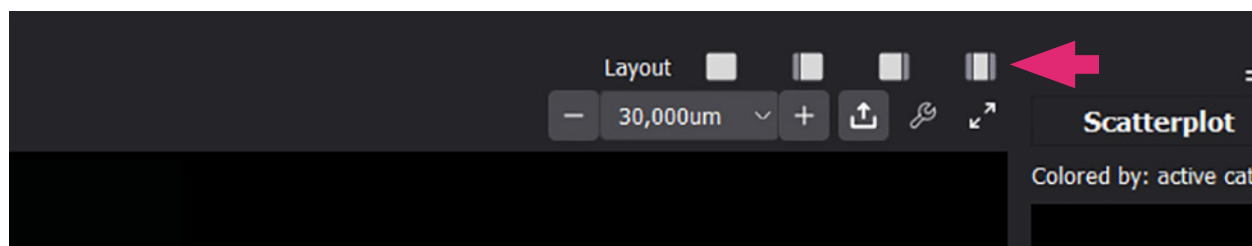
View

Floating panels surround the **spatial view window** (refer to [VIZUALIZER SOFTWARE USER INTERFACE OVERVIEW](#) for the default layout).

- Scatterplot
 - Spatial features
 - FPS measuring (FPS = frames per second)
 - Genes
 - Details
 - Selected items
- All panels except FPS measuring are shown by default after launching the MERSCOPE Vizualizer software.

To manage floating panels:

- ➡ **View** > toggle the applicable panel on and off.



- ➡ To change the relative layout of the **spatial view window** and panels, toggle among the layout icons above the top right of the **spatial view window**.
- ➡ To move a panel, move the cursor toward the top of the panel until the hand cursor appears. Click and drag to move the panel. Blue highlighting appears when hovering over a new location.
- ➡ To create layered floating panels, move the cursor toward the top of the panel until the hand cursor appears. Pick up the panel, drag and drop it over an existing floating panel (blue highlighting appears when hovering over the new location). The layered panels can be selected from tabs at the bottom of the window. To return to a classic layout, toggle among the layout icons above the top right of the **spatial view window**.
- ➡ To hide a panel, click X in the top right corner or toggle off from the **View** menu.
- ➡ To resize a panel, hover over the bottom of a panel until the resize cursor appears. Click and drag to resize.

- ➔ When the **FPS measuring** panel is open, click **Measure FPS** and a number appears after some time (>20 is reasonable, >60 is optimal).
- *If FPS is <5, the user experience may be improved by upgrading the graphics capabilities of the computer.*

Help

- ➔ Contact Vizgen help via support@vizgen.com.
- ➔ **About** > version information.
- ➔ About Qt (the C++ framework used for application development).
- ➔ Open the report folder.

Help > Archive Logs

To create a .ZIP folder to share with support@vizgen.com:

- ➔ **Help** > **Archive logs**.
- ➔ Describe the problem in the text box.
- ➔ Attach a screenshot, if applicable.
- ➔ Click **Save**.

The .ZIP folder will contain (as applicable):

- The application version.
- Information from the manifests of the last loaded experiments.
- Information from the manifests of the last assembled datasets.
- Debug information about application function.
- Crash dump files.

SPATIAL VIEW WINDOW

The MERSCOPE Vizualizer software has the capacity for two **spatial view windows**. The properties of each window are controlled independently. Each **spatial view window** displays a dataset in the experiment. If only one dataset is included during experiment assembly, only one **spatial view window** is visible by default.

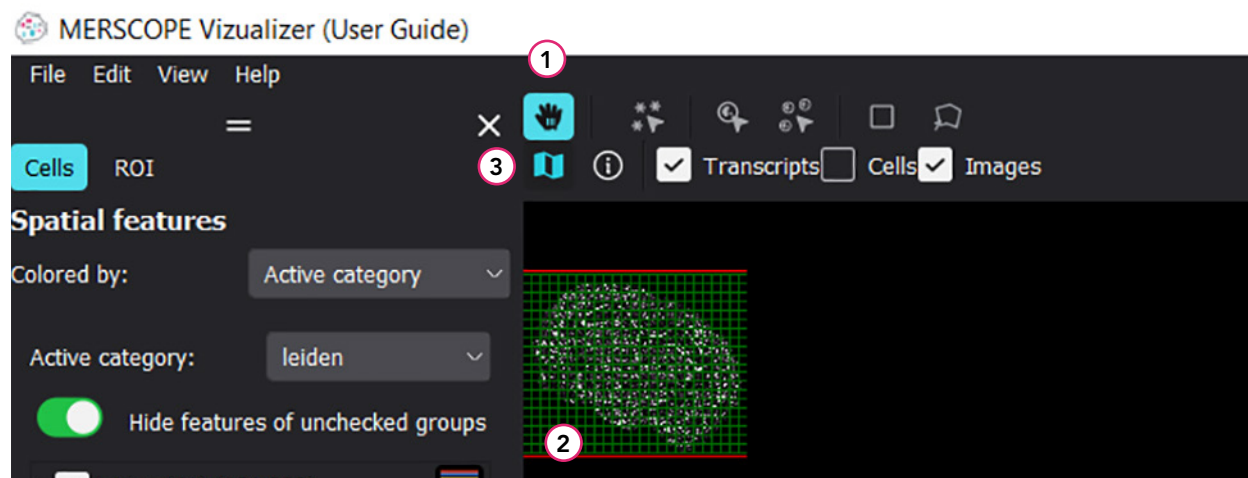
To open the second **spatial view window**, drag the right border of the **spatial view window** to the left. In the [Spatial Settings](#) menu of the new empty **spatial view window**, select the current dataset from the dataset menu (which is None by default). When two **spatial views windows** are open, it is possible to visualize the same dataset in the same or different locations.

In the **spatial view windows**, users can work with existing experimental data (cells, transcripts, images) and create custom cell groups and ROIs. Cell selection and cell group formation or ROI selection and ROI group formation in the **spatial view window** and **scatterplot** are synchronized.

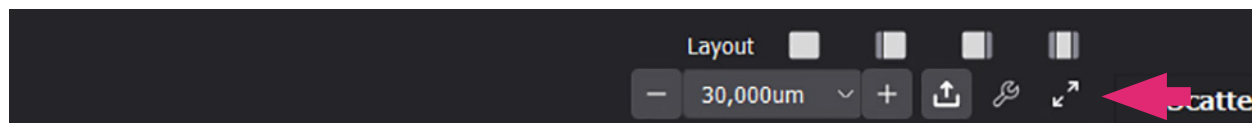
Tooltips

- ➡ Hover over a transcript, cell, or ROI to reveal a tooltip with information about the item.

Navigation and Views

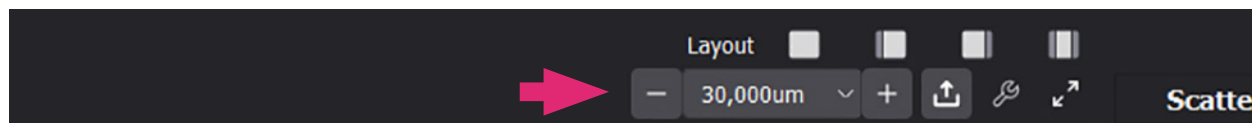


- ➡ In the **zoom/pan** cursor mode (hand icon) (1), click and drag the hand cursor in the **spatial view window** (and to move the content within the window).
- ➡ There is a mini map (2) - a small scale preview of the **spatial view window** - in the top left corner of the window. Click and drag to move the mini map boundary (red box) to navigate the content of the **spatial view window**. This feature is particularly useful when zoomed in.
- ➡ Toggle the mini map on and off via the map icon above the top left of the **spatial view window** (3).



- ➔ Change the content of the **spatial view window** to full screen and back by toggling the expand icon above the top right of the window.

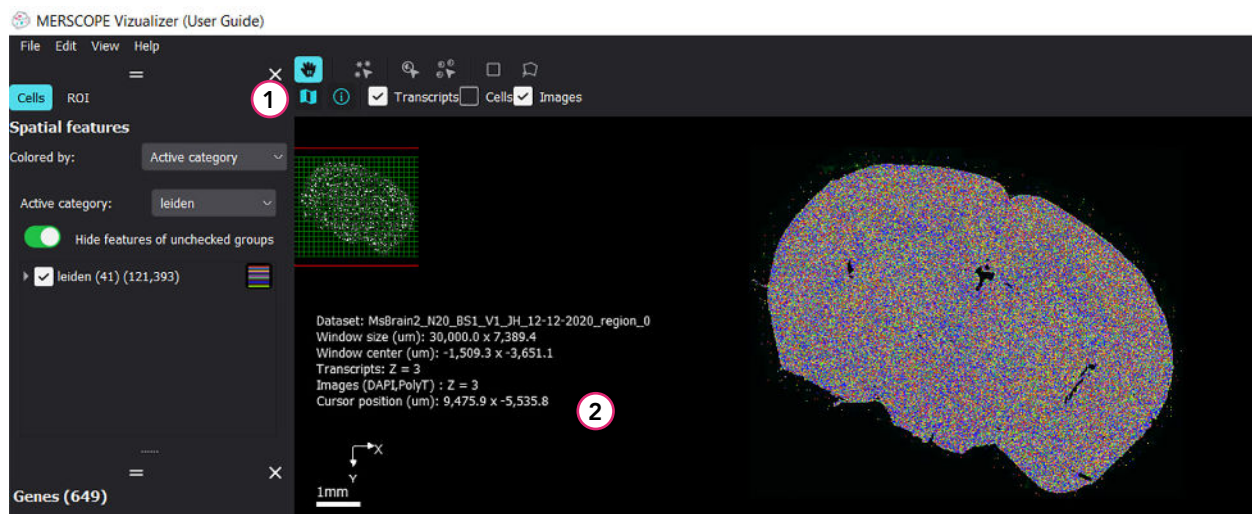
Zoom and Scale



To zoom in/out of the content in the **spatial view window**:

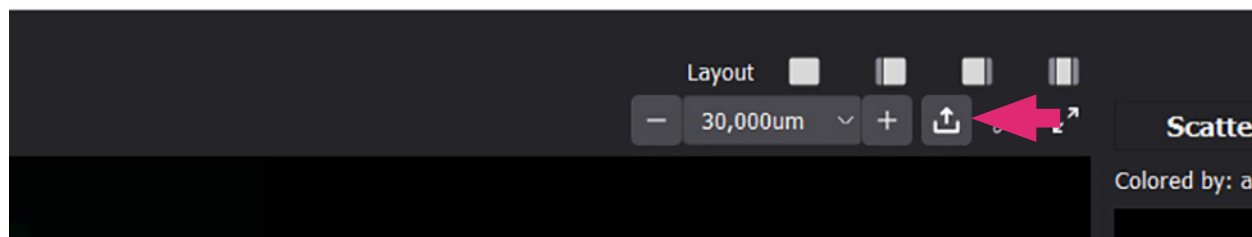
- ➔ Rotate the mouse wheel.
- ➔ Click the +/- buttons to either side of the scale box above the top right of the window.
- ➔ Type in an exact zoom level in microns in the scale box. The value corresponds to the width of the **spatial view window**. A scale bar is always present in the bottom left of the window.
- ➔ Select a zoom level in microns from the menu in the scale box.

Information Panel



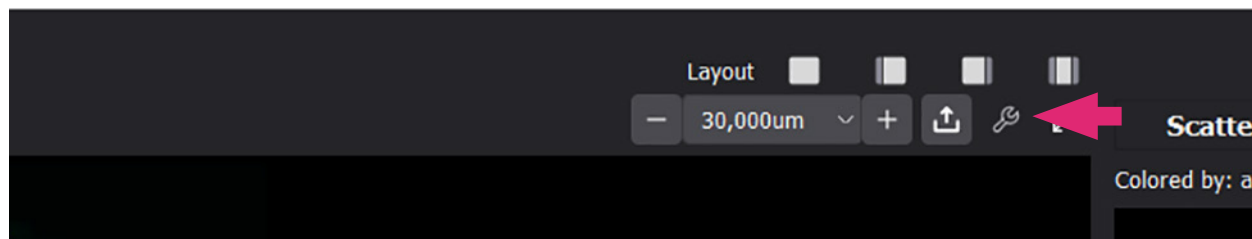
- ➔ Toggle a summary of properties of the data displayed in the window on and off via the information icon above the top left of the **spatial view window** (1).
- ➔ The **information panel** is displayed in the bottom left of the **spatial view window** (2), and includes the coordinates of the cursor in the **spatial view window**.

Export Image



- ➔ Click the export icon above the top right of the **spatial view window** to export a .png file of the **spatial view window**. A .txt file with the same file name with details of the data in the image file is also created.
- ➔ Changing the resolution factor of the .png file changes the resolution and size of the image file. The range of resolution factors depends on the rendering capabilities of the computer and the actual size of the exported **spatial view window** (a smaller window can be exported with a higher resolution factor).

Spatial Settings



To manage the properties of a **spatial view window**, toggle the **spatial settings** menu on and off via the tools icon above the top right of the **spatial view window**:

- ➔ Select the dataset visible in each spatial view window in the **Dataset** menu.
- ➔ To choose the z-plane visible in the **spatial view window**, select from the **Z-plane** menu. The active (selected) z-plane or all z-planes can be selected for each layer.
 - *Z-plane 0 includes the data at 1.5 microns from the MERSCOPE Slide. Z-planes 1, 2, 3, etc are at an additional fixed 1.5 microns away from the MERSCOPE Slide. The number of z-planes depends on the thickness of the sample defined during data acquisition.*
- ➔ Only the **transcripts** and **images** layers are visible in the **spatial view window** when an experiment is loaded. Additional layers are ROI feature sets, cells, and density.
- ➔ To toggle layers on and off in the **spatial view window**, check/uncheck the layer in the **spatial settings** menu or the shortlist of layers (**transcripts**, **cells** or the selected ROI feature set, **images**) above the top left of the **spatial view window**.

- ➡ To manage the order of layers, click the **Reorder layers** button and a dialog box appears:
 - The transcripts layer is always on top (first on the list) so that the user can always select transcripts in the **spatial view window**.
 - The **active feature set** is always listed second. Switching the **active feature set** in the **spatial features** panel automatically changes the layer list order. Selection tools always work on the **active feature set**.
 - For the remaining layers, drag and drop a layer into a different location to reorder layers.
- ➡ Each layer represents a particular type of spatial data. Cells and transcripts layers can be viewed across all z-planes or the active z-plane selected at the top of the **spatial settings** menu:
 - **Transcripts.** Shows the checked gene transcripts as dots. A slider controls the intensity. A separate slider controls the size of the transcripts.
 - **Cells.** Shows the polygons representing cells, identified during data processing. The slider controls the transparency.
 - **ROI feature sets.** Shows the ROIs in the set. Each ROI feature set has its own layer. The slider controls the transparency. ROIs span all z-planes and an ROI has the same boundary for all z-planes.
 - **Density.** Shows the density of checked gene transcripts. The slider controls the transparency.
 - **Images.** Images of the image channels, if checked (e.g., DAPI and PolyT). Up to 16 image channels can be displayed at the same time ([Edit > Manage Image Channels](#)). The **Images** slider is used to set the overall transparency for the images. Individual channel sliders are used to set minimum and maximum contrast thresholds for each channel. Only one z-plane (active) can be selected in the image layer.

Additional Settings (Under Spatial Settings Menu)

- ➡ To define the coordinate at the center of the **spatial view window**, enter X and Y values.
- ➡ To rotate the content in the **spatial view window**, adjust the **Rotate image** setting. To rotate clockwise, enter a positive number. To rotate counterclockwise, enter a negative number.
- ➡ Click the **Background color** palette to change the background color of the window.
- *If image channels are selected and/or not transparent, the background color change is not visible until the images are deselected in the **spatial settings** menu or from the layers check boxes above the top left of the **spatial view window**.*

DETAILS AND SELECTED ITEMS PANELS

Details Panel

The **details** panel displays details of a selected gene, cell, or ROI, including a list of genes expressed in a selected cell/ROI. Data are organized under spatial and heatmap headings. The information can be copied to clipboard using the copy icon at the top right of the **details** panel.

Selected Items Panel - Cells

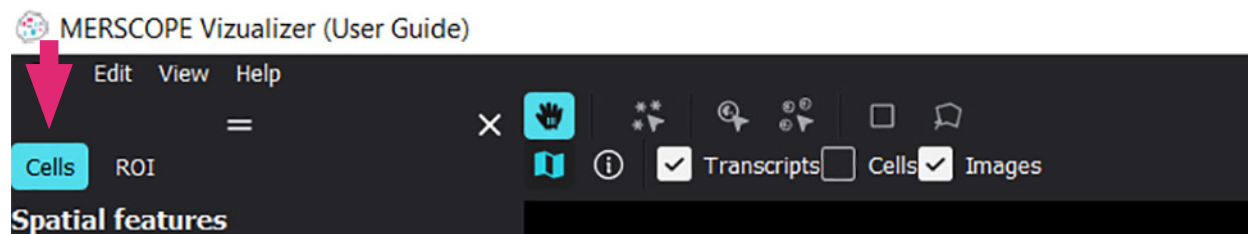
- ➔ The **selected items** panel indicates the **active feature set**, i.e., the **spatial feature set** selected in the **spatial features** panel.
- ➔ When the **cells feature set** is active in the **spatial features** panel, all cells selected in the **spatial features** panel, the **spatial view window**, **scatterplot**, and/or **heatmap** are listed in the **selected items** panel.
- ➔ Cell indexes and identification numbers of the selected cells are also listed. The number of selected cells is listed in parentheses next to the panel title **Selected items**.
- ➔ Click **Clear all** on the **selected items** panel to clear the selection in all panels. The **selected item** list is empty if no cells are selected (when in the **cells feature set**).
- ➔ Switching between **spatial feature sets** (cells or ROI) clears the **selected items** list.
- ➔ Individual cells in the list can be selected by clicking the cell in the list and the cell's location is identified by crosshairs in the **spatial view window**, **scatterplot**, and **heatmap**. The location is identified in the **heatmap** as long as the **Group by active category** slider is toggled off in the [Heatmap Toolbar](#).
- ➔ Alternatively, copy the cell name (e.g., 300948112130576190679118935843036609088) to clipboard. In the **selected items** panel, right-click (or Ctrl+V) to paste the cell name, and then select the cell in the single-item list to identify (crosshairs) the cell in the **spatial view window**, **scatterplot**, and **heatmap**.
- ➔ Cell groups ([Cell Groups](#)) can be created from this panel.

Selected Items Panel - ROI

- ➡ The **selected items** panel indicates the **active feature set**, i.e., the **spatial feature set** selected in the **spatial features** panel.
- ➡ When an **ROI feature set** is active in the **spatial features** panel, all ROIs selected in the **spatial features** panel, **spatial view window**, **scatterplot**, and/or **heatmap** are listed in the **selected items** panel.
- ➡ The number of selected ROIs in the group is listed in parentheses next to the panel title **Selected items**. Right-click on an ROI name to delete it.
- ➡ Click **Clear all** on the **selected items** panel to clear the selection in all panels. The **selected item** list is empty if no ROIs are selected (when in an **ROI feature set**).
- ➡ Switching between **spatial feature sets** (cells or ROI) clears the **selected items** list.
- ➡ Individual ROIs can be selected by clicking the ROI in the list and the center of the ROI is identified by crosshairs in the **spatial view window**, **scatterplot**, and **heatmap**. The location is identified in the **heatmap** as long as the **Group by active category** slider is toggled off in the [Heatmap Toolbar](#).
- ➡ ROI groups ([ROI Construction and ROI Groups](#)) can be created from this panel.

SPATIAL FEATURES PANEL - CELLS

Select the Cells Feature Set



The **cells feature set** and **ROI feature sets** ([Select an ROI Feature Set](#)) are individually addressable by selection of a feature set at the top of the **spatial features** panel. The **selected items** panel also indicates the **active feature set**, i.e., the **spatial feature set** selected in the **spatial features** panel. The **active feature set** is always displayed in front of other feature sets in the **spatial view window** and selection tools are only applicable to the **active feature set**.

Cells

The cell groups available in each cell category are listed in the **cells feature set** of the **spatial features** panel.

Each experiment contains two default cell categories:

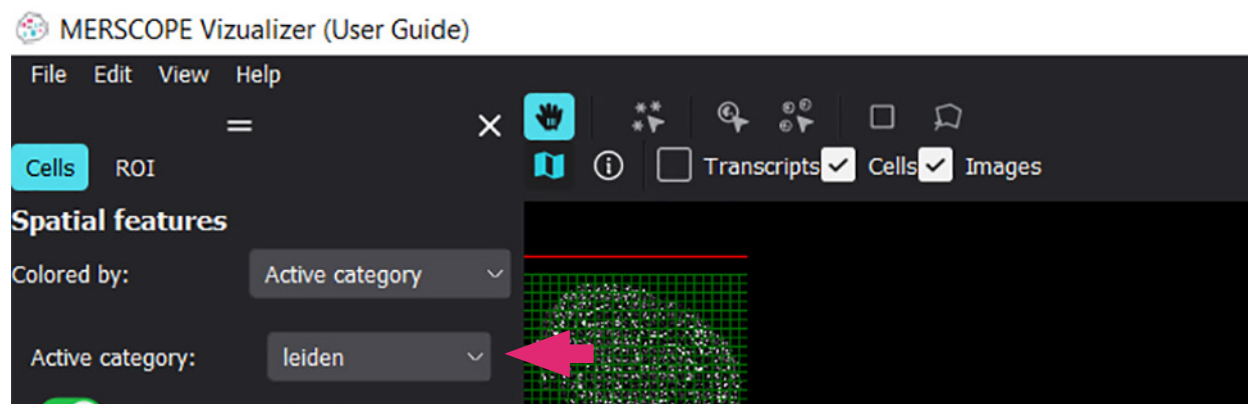
- **Datasets** is an immutable cell category. This category contains all the cells in each dataset.
- **Custom Cell Groups**. When an experiment is first assembled, a single cell group exists in this category (unclustered), which contains all the cells in an experiment. Custom cell groups can be created within this category.

In addition to default cell categories, additional cell categories can be imported with annotated data (e.g., Leiden).

It is possible to make a copy of custom cell groups ([Edit > Save Custom \[Feature Set\] Groups as New Copy](#)).

Active Category

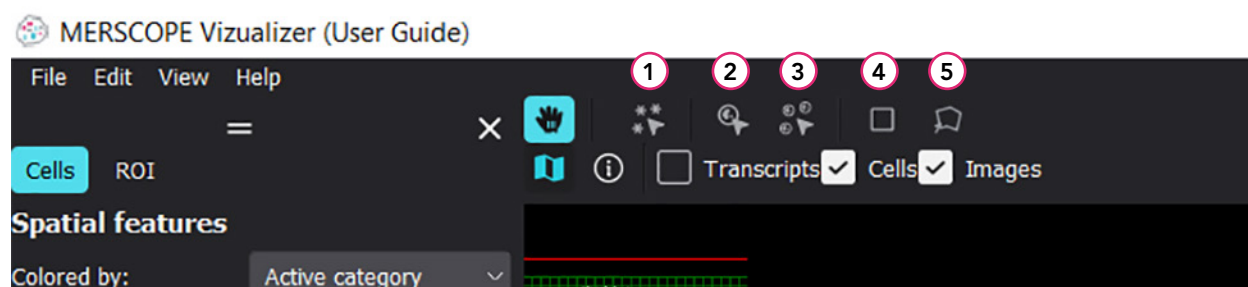
Select an **active category** from the menu near the top of the **spatial features** panel. The menu contains the default categories and any imported cell categories. The data of the **active category** is displayed in the **spatial view window**, **scatterplot**, and **heatmap**.



Selection Toolbar (Cells)

The **cells** layer in the **spatial view window** must be turned on to select cells and define cell groups. To toggle the **cells** layer on and off, check/uncheck the **cells** layer in the [Spatial Settings](#) menu or in the shortlist of layers above the top left of the **spatial view window** (available when the **cells** feature set is selected).

A selection toolbar is available above the top left of the **spatial view window**; the selection tools also work in the **scatterplot**.



- ➡ **Multi-transcript selection (1)** enables users to select all instances of a gene transcript at the same time. Click away from a selected transcript or press **Esc** and all [Checked Genes](#) transcripts reappear.
- ➡ **Single-feature selection (Cells) (2)** enables users to select a single cell. Click/click again to select/deselect an individual cell; click **Clear all** on the **selected items** panel, or press **Esc** to clear the selection. Select multiple cells by **Ctrl**-clicking single cells one-by-one. To deselect a cell from the selection, **Ctrl**-click in the **spatial view window**, **scatterplot**, or **heatmap** (as

long as the **Group by active category** slider is toggled off in the [Heatmap Toolbar](#)).

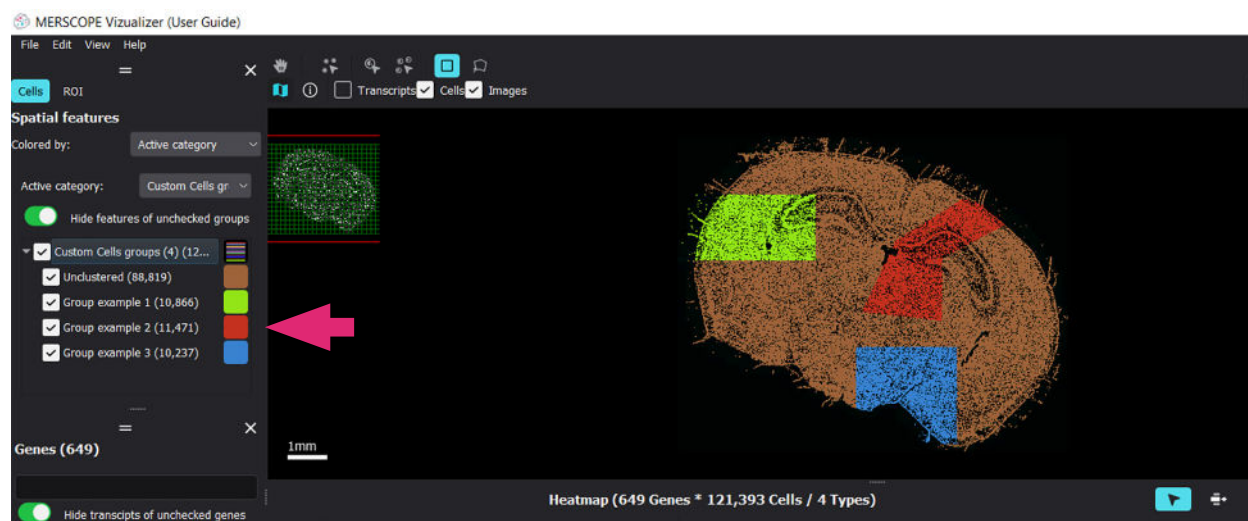
- ➡ **Multi-feature selection (Cells) (3)** enables users to select all cells in a cell group. Click/click again to select/deselect a cell group; click **Clear all** on the **selected items** panel to clear the selection. Select multiple cell groups by **Ctrl**-clicking cell groups one-by-one.
- ➡ **Rectangle selection (4)**. Drag and click to define a rectangle over the display to select cells at least partially within the rectangle. Press **Ctrl** and draw another rectangle to add cells to the previous selection. Draw another rectangle without **Ctrl** away from the original to reset the selection. Draw another rectangle within the first to select the subset of cells. Switch to the **single-feature selection** tool and click in a blank space in the **spatial view window**, click **Clear all** on the **selected items** panel, or press **Esc** to clear the selection.
- ➡ **Polygonal lasso selection (5)**. Click each point to draw a polygon over the display. Close the polygon to select the cells at least partially within the polygon. Press **Ctrl** and draw another polygon to add cells to the previous selection. Draw another polygon without **Ctrl** away from the original to reset the selection. Draw another polygon within the first to select the subset of cells. Switch to the **single-feature selection** tool and click in a blank space in the **spatial view window**, click **Clear all** on the **selected items** panel, or press **Esc** to clear the selection.

Cell Groups

Each cell can belong to either one cell group or no cell groups. A cell cannot belong to multiple cell groups.

To create a cell group:

- ➔ Select cells in the **spatial view window**, **scatterplot**, or **heatmap**. All selected cells are listed in the **selected items** panel. Cell indexes and identification numbers of the selected cells are also listed. The number of selected cells is listed in parentheses next to the panel title **Selected items**.
- ➔ Click on a cell in the **selected items** panel list and its location is identified by crosshairs in the **spatial view window** and **scatterplot**.
- ➔ To save selected cells as a cell group, enter a cell group name in the text box at the bottom of the **selected items** panel and click **Save group**. The cell group appears in the custom cells groups list in the **spatial features** panel. The number of cells in the group is listed in parentheses next to the cell group name.



- The newly created group always appears in the custom cell group category, regardless of which active category is selected.
- If a new cell group is created and the cell already belongs to a previously-defined cell group, the cell belongs to the newer group and is deleted from the previous group.
- After saving a cell group, the MERSCOPE Vizualizer software clears the selection.
- It is not possible to exclude (deselect) individual cells from a saved group.

To import a cell group:

- ➔ With an existing dataset open, export AnnData ([File > Export Data](#)) and export the **cells** feature set.

- ➔ Import AnnData into a Jupyter notebook ([Open Custom Cell Clustering Analysis](#)).

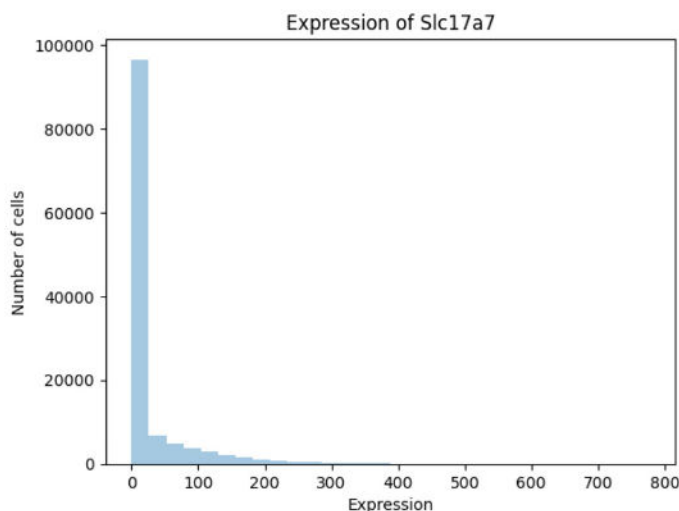
```
In [1]: import scanpy as sc

In [13]: import pandas as pd
import numpy as np

In [4]: adata = sc.read_h5ad('../MsBrain_custom_cell_group.hdf5')
adata

Out[4]: AnnData object with n_obs × n_vars = 121393 × 649
      obs: 'Custom Cells groups', 'Datasets', 'volume', 'center_x', 'center_y'
      uns: 'Custom Cells groups_colors', 'Datasets_colors'
```

- ➔ To create a new cell group, generate a new column of cell metadata.
- *In the example below, a new column of metadata (named new-group) was formed containing cells expressing the Slc17a7 gene at a high or low level.*



```
In [32]: ser_exp = adata.to_df()[gene_of_interest]
keep_cells = ser_exp[ser_exp > 30].index.tolist()

In [33]: adata.obs.loc[keep_cells, 'new-group'] = 'high'

In [34]: adata.obs['new-group'].value_counts()

Out[34]: new-group
low      98145
high     23248
Name: count, dtype: int64

In [35]: adata.write_h5ad('../MsBrain_new_group.hdf5')
```

- ➔ The Jupyter notebook saves this AnnData to a new .HDF5 file.
- ➔ Import this AnnData into the Vizualizer software ([File > Import Data](#)).
- ➔ Select the new column of cell metadata under the list of categories (e.g., new-group in the example above).
- ➔ The new cell category is available for selection and manipulation within the Vizualizer software.

Checked Cell Groups

- ➔ Select an **active category** from the menu in the **spatial view window**. The corresponding cell group tree structure appears below in the window. Click the arrow to the left of the parent cell group to expand the tree structure.
- ➔ Checked cell groups appear in the **spatial view window** and **scatterplot**, and are also included in the **heatmap**.
- ➔ To select all cells in a checked cell group, click on the cell group name in the **spatial features** panel. Selected cells are shown in the **spatial view window**, **scatterplot** and **heatmap**, and are also listed in the **details** panel.
- ➔ In the case of multilevel hierarchy, checking a parent group applies the same status to all child groups.

Unchecked Cell Groups

- ➔ Unchecked cells are hidden or colored gray in the **spatial view window** and **scatterplot**, and not included in the **heatmap**.
- ➔ Hide/unhide unchecked cell groups in the **spatial view window** and **scatterplot** by toggling the **Hide features of unchecked groups** slider in the **spatial features** panel.
- ➔ In the case of multilevel hierarchy, unchecking a parent group applies the same status to all child groups.

Cell Group Settings

- ➔ To color any cell group, left-click the color spot to the right of the group name and select a color.
 - *The color is applied to the cell group in the **spatial view window**, **scatterplot**, and **heatmap**.*
- ➔ Right-click on any cell group name to:
 - **Add/edit description** of the cell group.
 - **Reset color** back to the default color.
 - **Edit categories** to rename or remove imported categories.
 - **Remove group**. The option to remove any custom cell group created in the MERSCOPE Vizualizer software. Default or imported cell groups do not have the remove option. Cells from the removed group return to the unclustered group.
 - **Copy to clipboard**. Copies the cell identification numbers in the cell group to clipboard. It is pasted as a comma delimited list.
 - **Export**. Exports the cell identification numbers to a .CSV file format.

➡ Right-click on an **active category** name to:

- **Reset color** for all groups in the category back to the default colors.
- **Edit categories** to rename or remove imported categories.

Cell Color Options

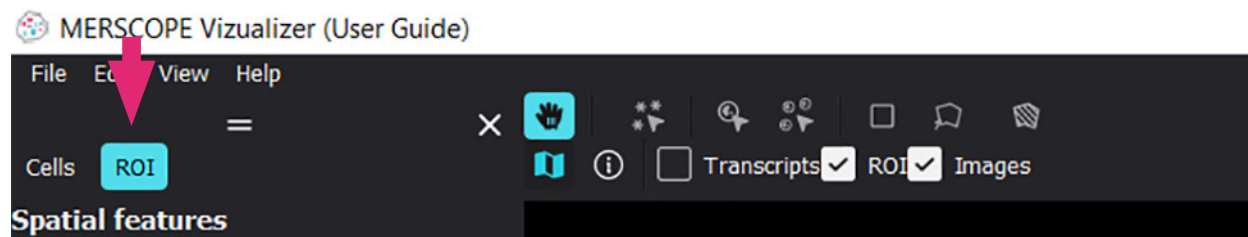
Cells can be colored according to cell category or a number of gene expression level characteristics. Cells can be colored according to:

- Active category
 - Count
 - Z-score
 - Normalized
 - Normalized z-score
- ➡ Cells are colored by **active category** by default. To change the coloring mode, select the option from the **Color by** menu at the top of the **spatial features** panel.
- *Active category applies the cell group colors defined by the color spot to the right of the cell group names.*
- ➡ Selection of an expression level characteristic reveals a color scheme menu.
- ➡ The three-color scheme based on minimum, center, and maximum values for each characteristic can be defined. Click on a color spot to change the color. Click on the center spot to convert to a two-color scheme (spot becomes transparent). Click again on the center spot to convert back to a three-color scheme and/or change the color.
- ➡ Adjust the **Brightness** slider to define the minimum and maximum thresholds for the characteristic.
- *The expression level characteristics for every cell are calculated dynamically based on the total expression of the checked genes. To color based on the expression of a single gene or a subset/group of genes, uncheck all genes and then check a single gene or subset/group of genes.*

SPATIAL FEATURES PANEL - REGIONS OF INTEREST

By default, there is one empty ROI feature set (named ROI) in the MERSCOPE Vizualizer software. ROI feature sets can be renamed or added via the [Edit > Manage ROI Sets](#) menu.

Select an ROI Feature Set

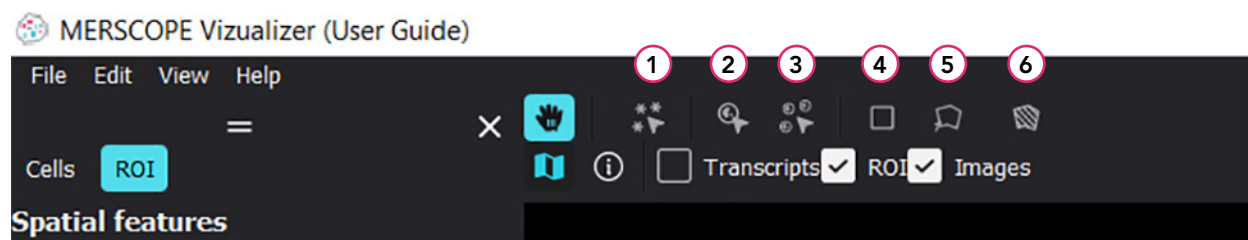


ROI feature sets and the cells feature set ([Select the Cells Feature Set](#)) are individually addressable by selection of a feature set at the top of the **spatial features** panel. The **selected items** panel also indicates the active feature set, i.e., the **spatial feature set** selected in the **spatial features** panel. The **active feature set** is always displayed in front of other feature sets in the **spatial view window** and selection tools are only applicable to the **active feature set**.

Selection Toolbar (ROIs)

An ROI feature set layer in the **spatial view window** must be turned on to see ROIs. To toggle ROI feature set layers on and off, check/uncheck an ROI feature set layer in the [Spatial Settings](#) menu or in the shortlist of layers above the top left of the **spatial view window** (available when an ROI feature set is selected).

When an ROI feature set is selected in the **spatial features** panel, a **Draw ROI polygon** icon (6) appears in the selection toolbar above the top left of the **spatial view window**; the selection tools also work in the **scatterplot**.



- ➡ **Multi-transcript selection** (1) enables users to select all instances of a gene transcript at the same time. Click away from a selected transcript or press **Esc** and all [Checked Genes](#) transcripts reappear.
- ➡ **Single-feature selection** ([Name of ROI feature set]) (2) enables users to select a single ROI. Click/click again to select/deselect an individual ROI; click **Clear all** on the **selected items** panel, or press **Esc** to clear the selection. Select multiple ROIs by **Ctrl-clicking** single ROIs

one-by-one. To deselect a ROI from the selection, **Ctrl-click** in the **spatial view window**, **scatterplot**, or **heatmap** (as long as the **Group by active category** slider is toggled off in the [Heatmap Toolbar](#)).

- ➔ **Multi-feature selection** ([Name of ROI feature set]) (3) enables users to select all ROIs in an ROI group. Click/click again to select/deselect an ROI group; click **Clear all** on the **selected items** panel to clear the selection. Select multiple ROI groups by **Ctrl-clicking** ROI groups one-by-one.
- ➔ **Rectangle selection** (4). Drag and click to define a rectangle over the display to select ROIs at least partially within the rectangle. Press **Ctrl** and draw another rectangle to add ROIs to the previous selection. Draw another rectangle without **Ctrl** away from the original to reset the selection. Draw another rectangle within the first to select the subset of ROIs. Switch to the **single-feature selection** tool and click in a blank space in the **spatial view window**, click **Clear all** on the **selected items** panel, or press **Esc** to clear the selection.
- ➔ **Polygonal lasso selection** (5). Click each point to draw a polygon over the display. Close the polygon to select the ROIs at least partially within the polygon. Press **Ctrl** and draw another polygon to add ROIs to the previous selection. Draw another polygon without **Ctrl** away from the original to reset the selection. Draw another polygon within the first to select the subset of ROIs. Switch to the **single-feature selection** tool and click in a blank space in the **spatial view window**, click **Clear all** on the **selected items** panel, or press **Esc** to clear the selection.
- ➔ **Draw ROI polygon** (6). Click each point to draw a polygon over the display. Close the polygon and a dialog box appears.

ROI Construction and ROI Groups

- ➔ To draw an ROI, use the **Draw ROI Polygon** tool. Click each point to draw a polygon over the display. Close the polygon and a dialog box appears. Press **Esc** before closing the polygon to reset the drawing.
- ➔ Enter a name for the newly drawn ROI.
- ➔ Select the group that the ROI should be added to or create a new group. To create a new group, overwrite the default group name in the **ROI group** box. To select from existing groups, click in the ROI group box and a list of existing ROI groups appears.
- ➔ Click **Cancel** to remove the ROI just drawn.
- *When an **ROI feature set** is selected in the **spatial features** panel, all ROIs in any selected group are listed in the [Selected Items Panel - ROI](#). The number of selected ROIs in the group is listed in parentheses next to panel title **Selected items**. Individual ROIs can be selected by clicking the ROI in the list and the center of the ROI is identified by crosshairs in the **spatial view window**.*

- To save selected ROIs as a new ROI group, enter an ROI group name in the text box at the bottom of the **selected items** panel and click **Save group**. The ROI group appears in the custom ROI groups list in the **spatial features** panel. The number of ROIs in the group is listed in parentheses next to the ROI group name.
- To delete an ROI, right-click on the ROI name in the **selected items** panel.
- If a new ROI group is created and an ROI already belongs to a previously-defined ROI group, the ROI belongs to the newer group and is removed from the previous group. If the ROI group is empty (no ROIs) after moving an ROI, the group will disappear from the list in the **spatial features** panel.
- After saving an ROI group, the MERSCOPE Vizualizer software clears the selection.
- It is not possible to exclude (deselect) individual ROIs from a saved group.

Checked ROI Groups

- ➔ Checked ROIs appear in the **spatial view window** and **scatterplot**, and are included in the **heatmap**.
- ➔ To select all ROIs in a checked ROI group, click on the ROI group name in the **spatial features** panel. The ROIs in the group are listed in the **selected items** panel.
- ➔ In the case of multilevel hierarchy, checking a parent group applies the same status to all child groups.

Unchecked ROI Groups

- ➔ Unchecked ROIs are hidden or colored gray in the **spatial view window** and **scatterplot** and not included in the **heatmap**.
- ➔ Hide/unhide unchecked ROI groups by toggling the **Hide features of unchecked groups** slider in the **spatial features** panel.
- ➔ In the case of multilevel hierarchy, unchecking a parent group applies the same status to all child groups.

ROI Group Settings

- ➔ To color any ROI group, left-click the color spot to the right of the group name and select a color.
- The color is applied to the ROI group in the **spatial view window**, **scatterplot**, and **heatmap**.
- ➔ Right-click on any ROI group name to:
 - Add/edit description of the ROI group.

- **Reset color** back to the default color.
- **Edit categories** to rename or remove imported categories.
- **Remove group.** The option to remove any custom ROI group created in the MERSCOPE Vizualizer software. Default or imported ROI groups do not have the remove option. ROIs from the removed group return to the unclustered group.
- **Copy to clipboard.** Copies the ROI names in the ROI group to clipboard. It is pasted as a comma delimited list.
- **Export.** Exports the ROI names to a .CSV file format.

➡ Right-click on an **active category** name to:

- **Reset color** for all groups in the category back to the default colors.
- **Edit categories** to rename or remove imported categories.

ROI Color Options

ROIs can be colored according to ROI category or a number of gene expression level characteristics. ROIs can be colored according to:

- Active category
 - Count
 - Z-score
 - Normalized
 - Normalized z-score
- ➡ ROIs are colored by **active category** by default. To change the coloring mode, select the option from the **Color by** menu at the top of the **spatial features** panel.
- *Active category applies the ROI group colors defined by the color spot to the right of the ROI group names.*
- ➡ Selection of an expression level characteristic reveals a color scheme menu.
- ➡ The three-color scheme based on minimum, center, and maximum values for each characteristic can be defined. Click on a color spot to change the color. Click on the center spot to convert to a two-color scheme (spot becomes transparent). Click again on the center spot to convert back to a three-color scheme and/or change the color.
- ➡ Adjust the **Brightness** slider to define the minimum and maximum thresholds for the characteristic.
- *The expression level characteristics for every ROI are calculated dynamically based on the total expression of the checked genes. To color based on the expression of a single gene or a subset/group of genes, uncheck all genes and then check a single gene or subset/group of genes.*

SCATTERPLOT

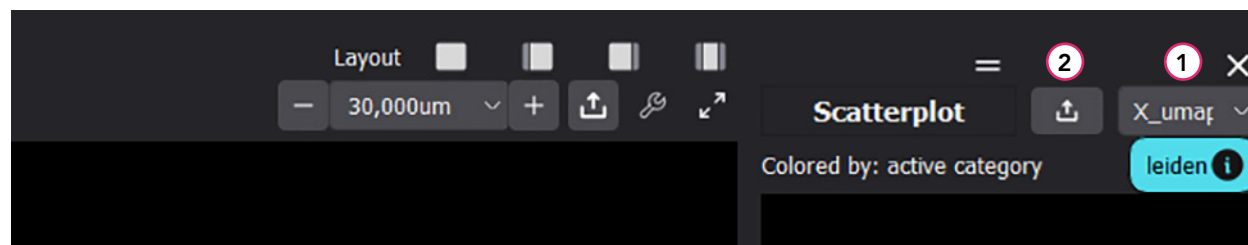
The MERSCOPE Vizualizer software has a single **scatterplot**. The **scatterplot** can display two-dimensional arrays, such as cells in dimensionality reduced gene expression space (e.g., UMAP/tSNE) or other user-imported two-dimensional arrays (e.g., cell volume versus transcript count). Clustering modes are imported with annotation data (AnnData). The **active feature set** is displayed in the **scatterplot**. If features are colored by expression (count, Z-score, normalized, Z-score normalized) in the **spatial features** panel, the **active feature set** is indicated at the top right of the **scatterplot**. If there are no two-dimensional data for an **ROI feature set**, the **scatterplot** will be empty.

- Only cells or ROIs from the experiment that have imported annotation are shown in the **scatterplot**. E.g., if some cells or ROIs were filtered during clustering, they are not displayed in the **scatterplot**.

Translate and Zoom

- ➔ In the **zoom/pan** cursor mode (hand icon) from above the **spatial view window** ([Navigation and Views](#)), click and drag the cursor in the **scatterplot** to translate the content.
- ➔ To zoom in/out of the content in the **scatterplot**, rotate the mouse wheel.

Select Array



- ➔ If multiple arrays were imported, select the array visible in the panel from the menu above the **scatterplot** (1).

Export Image

- ➔ Click the export icon (2) above the **scatterplot** to export a .png file of the content.
- ➔ Changing the resolution factor of the .png file changes the resolution and size of the image file. The range of resolution factors depends on the rendering capabilities of the computer and the actual size of the exported **spatial view window** (a smaller window can be exported with a higher resolution factor).

Selection Toolbar (Cells or ROI)

Selection toolbar functions can be applied to the **scatterplot** in the same way as in the **spatial view window**. Refer to [Selection Toolbar \(Cells\)](#) or [Selection Toolbar \(ROIs\)](#) for more information.

Cell groups or ROI groups can be created in the **scatterplot** in the same way as in the **spatial view window**. Refer to [Cell Groups](#) or [ROI Construction and ROI Groups](#) for more information.

GENES TREE

The **genes** panel controls the representation of gene products - transcripts - in the MERSCOPE Vizualizer software.

In the **spatial view window**, transcripts are represented by dots and all transcripts that are products of the same gene have the same color.

The **transcripts** layer in the **spatial view window** must be turned on to see transcripts. The transcripts layer is visible by default when an experiment is loaded. To toggle the **transcripts** layer on and off, check/uncheck the **transcripts** layer in the [Spatial Settings](#) menu or in the shortlist of layers above the top left of the **spatial view window**.

Checked Genes

- ➡ All genes included in an experiment are listed in the **genes** panel. The number of genes in the gene panel is listed in parentheses next to the software panel title **Genes** and next to **All** in the list. The number of transcripts of each gene in the experiment (both inside and outside cell boundaries) is listed in parentheses next to the gene name. The number of genes in [Gene Groups](#) are listed in parentheses next to the gene group.
- ➡ To visualize all transcripts of a gene/gene group, check the box next to the gene/gene group name.
- ➡ Checked gene transcripts appear in the **spatial view window**.
- ➡ Checked genes are displayed in the **heatmap**.
- ➡ Selected genes are also highlighted in the **heatmap** ([Heatmap Highlighting](#)). Click on the gene name in the **genes** panel to select the gene.
- ➡ In the case of multilevel hierarchy, checking a parent group applies the same status to all child groups.

Unchecked Genes

- ➡ Unchecked genes are hidden or colored gray in the **spatial view window** and **scatterplot**, and not included in the **heatmap**.
- ➡ Hide/unhide unchecked genes by toggling the **Hide transcripts of unchecked genes** slider in the **genes** panel.
- ➡ In the case of multilevel hierarchy, unchecking a parent group applies the same status to all child groups.

Gene Color Settings

- ➡ To change the color of gene transcripts, left-click the color spot to the right of the gene name and select a color.

Gene Settings

- ➡ Right-click on any gene name/color spot to see a menu to:
 - **Add/edit description** of the gene.
 - **Move** to another gene group - select the gene group from the menu or create a new group.
 - **Reset color** back to the default color.
 - **Copy to clipboard.** Copies the gene group, gene name, and color information in the gene to clipboard. It includes any description of the gene. It is pasted as a comma delimited list. The gene must be highlighted (left-click) prior to right-click to copy (otherwise information is not copied).
 - **Export.** Exports the gene group, gene name, gene description, and color information to a .CSV file format. The gene must be highlighted (left-click) prior to right-click for export (otherwise the exported file will not contain information).
 - **Import.** Gene groups, description, and color can be imported from a .CSV file. Click **Import** to upload a .CSV file, which must contain 4 columns: gene group, gene name, gene description, gene color; gene names are immutable. It is recommended to first export a .CSV file, edit it, and then import it.

Gene Highlighting

- ➡ Left-click a gene or gene group to highlight it in the genes tree.
- ➡ Ctrl-click to highlight multiple genes.
- ➡ Right-click to move selected gene(s) to another group or create a new group.
- ➡ Highlighted genes are marked by a blue bar in the genes tree.
- ➡ Use the up/down arrow keys to move highlighting up/down in the genes tree.
- ➡ Unchecked genes are not highlighted if **Hide transcripts of unchecked genes** is toggled on.
- ➡ In the **spatial view window**:
 - Highlighted gene(s) are colored.
 - Non-highlighted genes are gray.
- ➡ In the **heatmap**:
 - Highlighted gene(s) are marked by a blue bar ([Heatmap Highlighting](#)).
- ➡ A gene must be checked in order to be shown in the visualization regardless of highlighting.

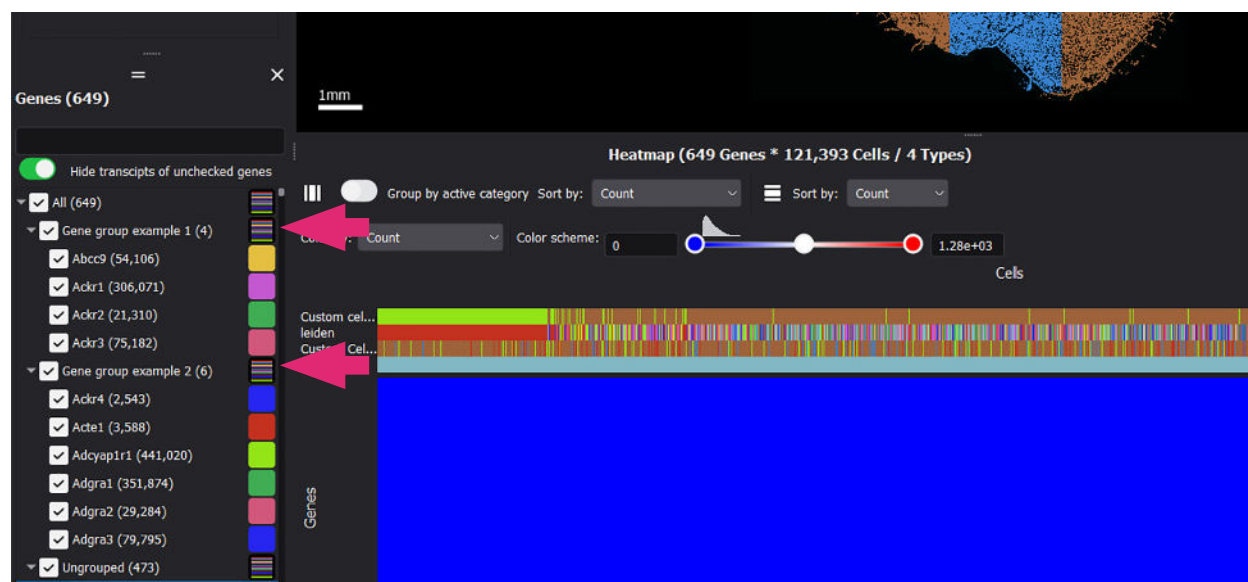
Gene Groups

Each gene can belong to either one gene group or no gene groups. A gene cannot belong to multiple gene groups.

Genes without a group are designated ungrouped. A gene group can be created from checked genes, from highlighted genes, or an empty gene group can be created. If a gene group is deleted, the genes in the group automatically return to the ungrouped status. It is not possible to delete a gene from the **genes** panel.

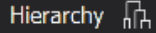
To create a gene group from checked genes:

- ➔ Check genes in the **genes** panel.
- ➔ To save checked or highlighted genes as a gene group, enter a gene group name in the text box at the bottom of the **genes** panel and click **Save**. The gene group appears in the tree structure in the **genes** panel. The number of genes in the group is listed in parentheses next to the gene group name. The number of transcripts of each gene in the experiment (both inside and outside cell boundaries) is listed in parentheses next to the gene name.



- ➔ Click the arrow to the left of the parent gene group to expand the tree structure.
- If a new gene group is created and the gene already belongs to a previously-defined gene group, the gene belongs to the newer group and is deleted from the previous group.
- ➔ To create an empty gene group, ensure no genes are selected, enter a gene group name in the text box at the bottom of the **genes** panel and click **Save**.
- An empty gene group can be useful if users want to create a gene group in a stepwise fashion, adding one gene at a time, for example.
- ➔ Gene groups can be created by right-click from highlighted genes (Move to > Create new

group).

- ➔ Gene groups can be created from the **heatmap** by clicking on an individual cluster or by clicking the **hierarchy** button  in the **genes** panel when hierarchical clustering is selected in **heatmap** toolbar.
- ➔ Gene groups can be highlighted in the **heatmap**.

Search for Genes

- ➔ Use the search bar at the top of the **genes** panel to search for genes in all groups.

Gene Group Settings

- ➔ Right-click on any gene group name/color spot to see a gene group menu to:
 - **Color by group(s)**. Changes all genes in the same group to the same color. To change the color of a gene group, left-click the color spot to the right of the gene group name and select a color.
 - **Reset color** back to the default color.
 - **Delete group**. The option to delete any gene group created in the MERSCOPE Vizualizer software. When a gene group is deleted, the genes in the group return to the ungrouped status.
 - **Copy to clipboard**. Copies the gene group, gene name, and color information of all genes in the gene group to clipboard. It is pasted as a comma delimited list. The gene group must be highlighted (left-click) prior to right-click to copy (otherwise information is not copied).
 - **Export**. Exports the gene group, name, gene description, and color information to a .CSV file format. It is possible to save the different types of genes grouping, e.g., grouping based on hierarchical clustering, DEG analyses, etc. The gene group must be highlighted (left-click) prior to right-click for export (otherwise the exported file will not contain information).
 - **Import**. Gene groups, description, and color can be imported from a .CSV file. Click **Import** to upload a .CSV file, which must contain 4 columns: gene group, gene name, gene description, gene color; gene names are immutable. All genes from gene panel will be regrouped according to the information in the .CSV file. It is recommended to first export a .CSV file, edit it, and then import it.

HEATMAP

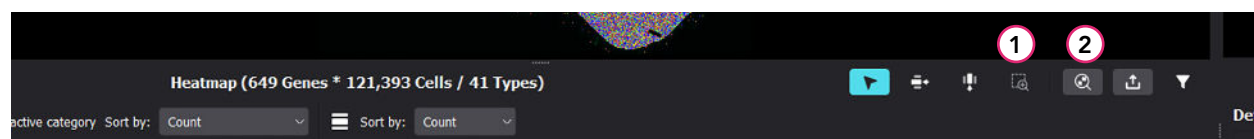
The MERSCOPE Vizualizer software has a single **heatmap**, where users can view gene expression data according to spatial feature categories and groups. All cells from all datasets are shown in the **heatmap** and **scatterplot**. To view cells from a specific dataset(s) in an experiment assembled from multiple datasets, select **Datasets** in the [Active Category](#) menu in the **spatial features** panel and uncheck any listed dataset to hide the cells from that dataset.

Navigation and Selection



- ➔ In the **zoom/pan** cursor mode (arrow icon) (1), click and drag the hand cursor in the heatmap to translate the content of the window.
- ➔ In the **gene selection** cursor mode (row icon) (2), click a gene in the heatmap to select it. Information on the selected gene appears in the **details** panel.
- ➔ In the **feature selection** cursor mode (column icon) (3), click a feature in the heatmap to select it. Information on the selected feature appears in the **selected items** panel.

Zoom and Scale



- ➔ To zoom in/out of the content in the **heatmap**, rotate the mouse wheel.
- ➔ In the **zoom to rectangle** mode (1), draw a rectangle over the **heatmap** to expand the contents of the rectangle.
- ➔ Use the **reset zoom** mode (2) to visualize the entire heatmap.

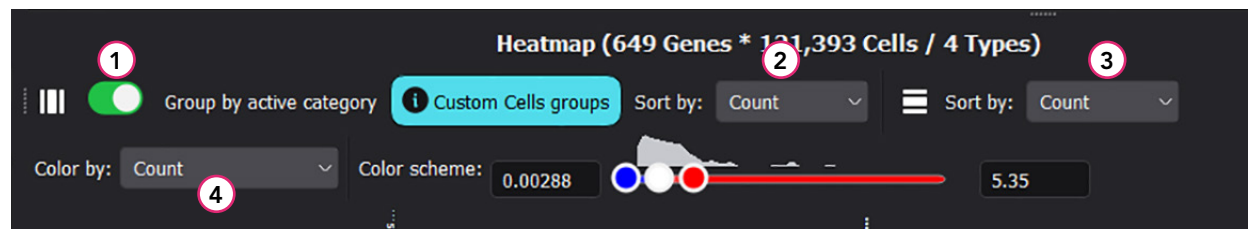
Export




- ➔ Click the export icon to export a .png file of the **heatmap** or export the filtered expression matrix to a .CSV file or .PARQUET file.

Heatmap Toolbar

The **spatial feature set** (cells or ROI) selected in the **spatial features** panel is visible in the **heatmap** and the data are ordinarily displayed per feature. Only [Checked Cell Groups](#) or [Checked ROI Groups](#), and [Checked Genes](#) are visible in the heatmap.



- ➔ The **active category** selected in the **cells/ROI feature set** is displayed in the heatmap. To display data according to cell/ROI groups in a category, toggle on the **Group by active category** slider (1).
- All cell categories are shown in the heatmap bar (at the top) in the order they appear in the active category list in the spatial features panel. When the **Group by active category** slider (1) is toggled on, only the active category is shown in the heatmap bar.
- ➔ Cells/ROIs can be sorted according to count or by count within each category (count+category) via the feature **sort by** menu (when the **cells feature set** is selected) (2).
- ➔ Genes can be sorted according to count, variance, or hierarchical clustering via the gene **sort by** menu (3). If hierarchical clustering is selected, it is possible to create gene groups via the **hierarchy** button in the **genes** panel ([Gene Groups](#)).
- ➔ When the **Group by active category** slider (1) is toggled on, the feature **sort by** menu (2) enables sorting by hierarchical clustering or count. The sliders on each axis of the **heatmap** (bottom left for genes and top right for category) adjust the level of the hierarchical dendrograms that are displayed as brackets on either axis (sliders are only visible when hierarchical sorting is selected).
- If selected genes or feature groups are changed, orange warning triangles  appear next to the **sort by** menus and the dendrograms appear orange. Click the **recalculate hierarchical clustering button** to the top left of the **heatmap** (indicated by an orange warning triangle/circular arrow) to adjust the clustering according to the new selections.
- ➔ **Spatial features** (cells or ROI) in the **heatmap** can be colored according to gene expression level. **Spatial features** can be colored according to count, Z-score, normalized, and normalized z-score (4).
- ➔ The three-color scheme based on minimum, center, and maximum values for each characteristic can be defined. Click on a color spot to change the color. Click on the center spot to convert to a two-color scheme (spot becomes transparent). Click again on the center spot to convert back to a three-color scheme and/or change the color.

- ➡ Adjust the slider to define the minimum and maximum thresholds for the characteristic.

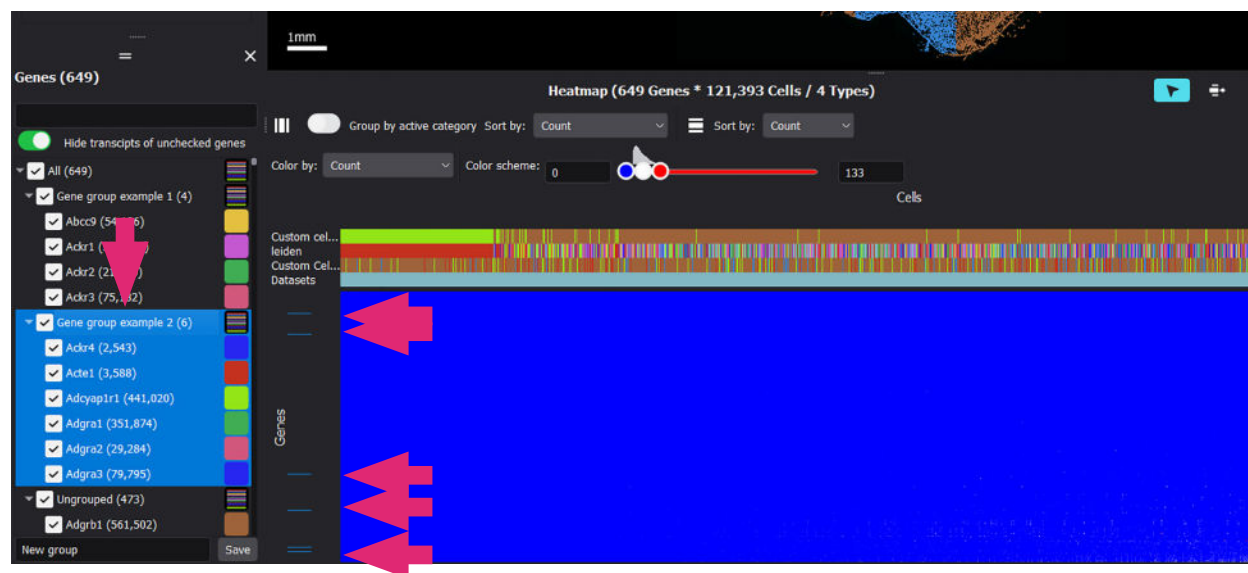
Heatmap Settings

To manage properties of the **heatmap**, toggle the **heatmap settings** menu on and off via the funnel icon above the top right of the **heatmap**.



- ➡ Adjust the **Variance of highly variable genes** slider to set a cutoff for genes to be displayed in the **heatmap**, and click **Apply**. When adjusting the slider, the number of kept genes appears under the box, next to an information icon.
 - After clicking **Apply**, the genes below cutoff are unchecked in the **genes** panel. To reset the genes included in the **heatmap**, re-check the genes in the **genes** panel.
- ➡ Adjust the **Z-score of differentially expressed genes (DEG)** slider to set a cutoff for genes to be displayed in the heatmap, and click **Apply**. When adjusting the slider, the number of kept genes appears under the box next to an information icon.
 - Only checked gene groups are taken into account in DEG calculation.
 - At least two cell groups or ROI groups should be checked for Z-score or DEG calculation.
 - DEG calculation for a large number of groups may take some time (up to 60 sec).
 - If the **Z-score of differentially expressed genes** slider is not visible, click **Recalculate DEG** and the slider will appear. This may take some time.
 - After clicking **Apply**, the genes below cutoff are unchecked in the **genes** panel. To reset the genes included in the **heatmap**, re-check the genes in the **genes** panel.

Heatmap Highlighting



Locations of selected and checked items are highlighted in the heatmap by a blue bar on the applicable axis. Items must be checked to be highlighted (gene group member highlighting illustrated). Also refer to [Gene Highlighting](#).

Highlighting on the y-axis (genes):

- Individual genes are highlighted when a gene transcript is selected in the **spatial view window**.
- Individual genes are highlighted when a gene is selected in the **genes** panel.
- Gene group members are highlighted when a gene group is selected in the **genes** panel.
- Individual genes are highlighted when a gene name (label) on the left side of the **heatmap** is selected.
- Use **Ctrl-click** to select multiple genes.

Highlighting on the x-axis (cells or ROIs):

- Individual cells or ROIs are highlighted when a cell or ROI name (label) at the top of the **heatmap** is selected. Use **Ctrl-click** to select multiple individual cells or ROIs. The selected (and highlighted) cells or ROIs are listed in the **selected items** panel.
- Individual cells or ROIs are also highlighted when a cell or ROI is selected in the **spatial view window** or **scatterplot**.
- Cell or ROI group members are highlighted when selected in the **spatial features** panel, or in the **spatial view window** or **scatterplot** using the multi-feature selection tool.

Cell/ROI names and gene names can be copied from **heatmap** axes by right-click on the name.