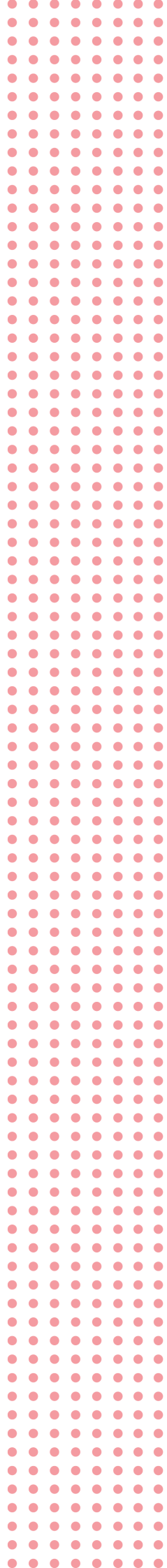
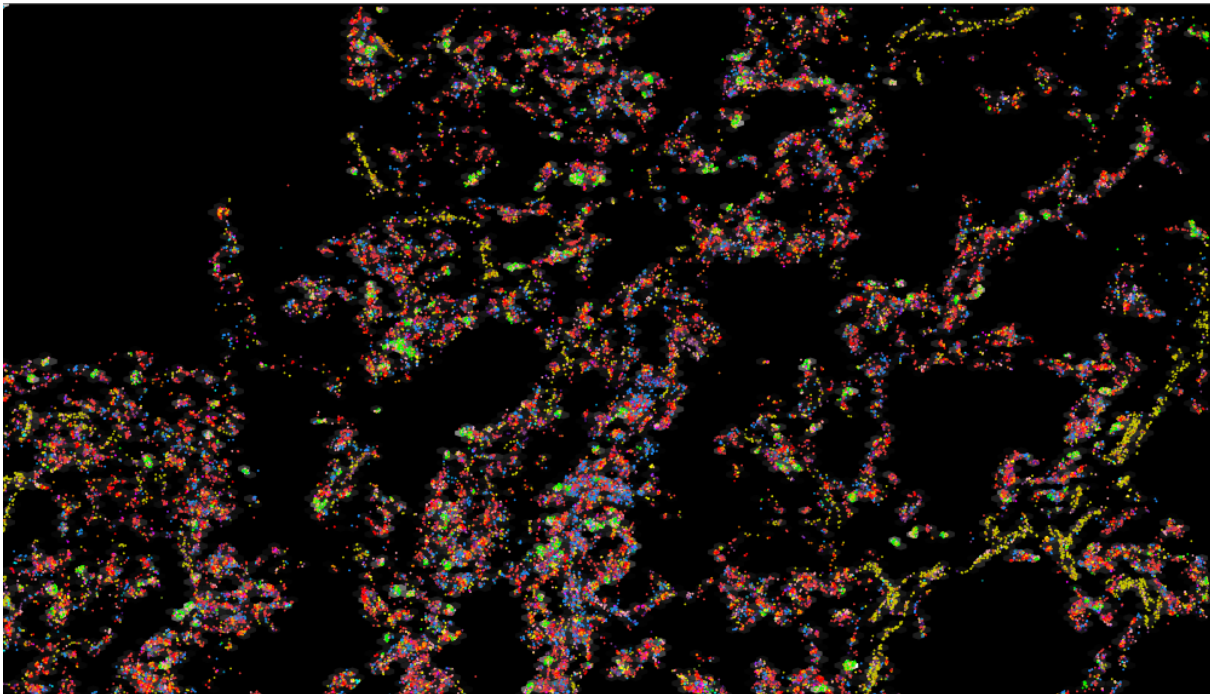




Automated Customer Report



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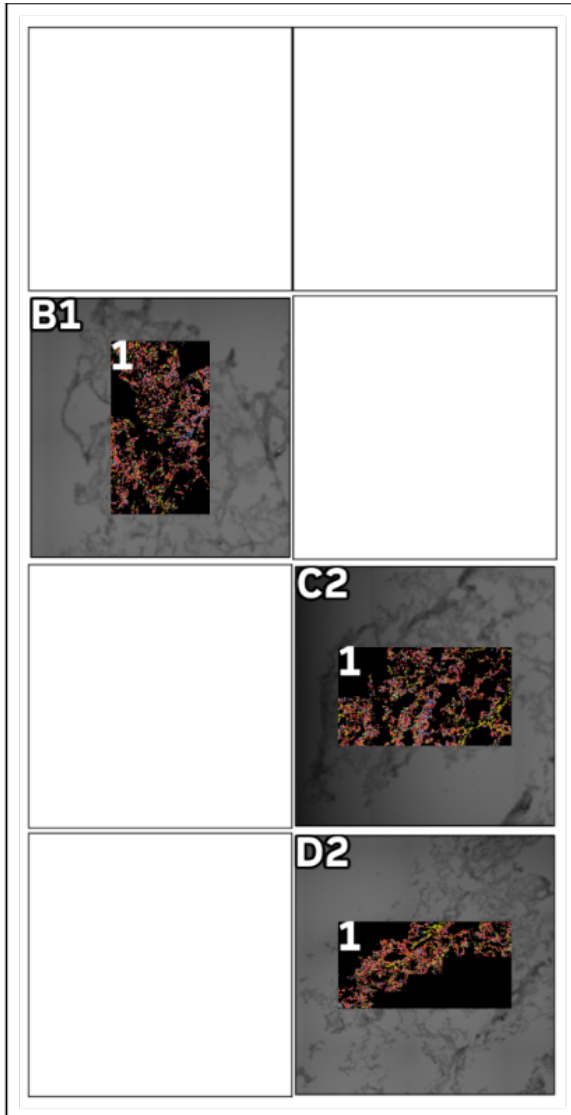
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Project ID: 00008

Slide (internal ID): slide879

Each experiment consists of one microscopy slide with up to eight samples which can be used to image multiple regions of interest (ROIs). The images on the left show the samples in grayscale with their label in the upper left corner. The colored overlays are the imaged ROIs. They will hence be labeled as indicated in the table to the right.



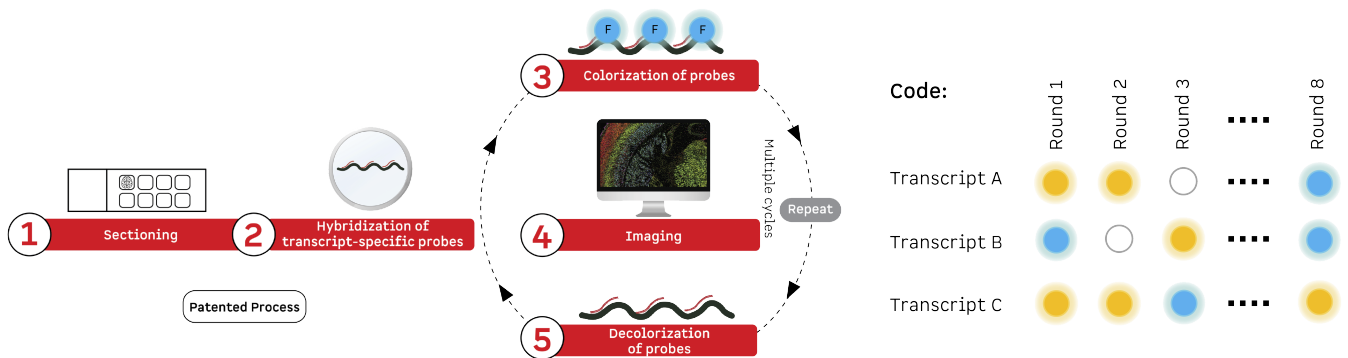
List of ROIs in samples	
Sample	ROIs
B1	B1-1
C2	C2-1
D2	D2-1

1.1 // Gene panel

ACTG2	FCN1
AGER	FGFR4
AGR2	FOLR1
ALDH2	GPC3
C1QB	IL7R
C3	KRT8
CALB2	LAMP3
CAV1	LUM
CD3D	MARCO
CD34	MYH11
CFD	PECAM1
CHI3L1	PTPRC
CSTA	SERPINF1
EDN1	SFTPD
EPCAM	SPINT2
FABP4	TIMP3
FBLN1	VCAN

1.2 // Molecular Cartography™ workflow overview

Molecular Cartography is based on combinatorial single-molecule fluorescent *in situ* hybridization (sm-FISH). We hybridize several tens of transcript-specific probes per target RNA, for up to a 100 RNA species per sample. Through a proprietary coloring and de-colorizing chemistry our technology accurately identifies each transcript by decoding a barcode specific to each transcript-species using several imaging rounds. Due to this approach, Molecular Cartography offers unprecedented specificity and sensitivity with one spot being one transcript which allows digital quantification. During the transcriptomics assay the tissue remains intact and can be processed for downstream analysis such as dye-labeling or immunohistochemistry.



2.1 // High abundance spatially distributed genes

Figure 1 highlights four selected genes in each ROI. In order to provide a clearer image, the colors of these genes may differ in subsequent ROIs. The genes are selected by their standard deviation between areas of the respective ROI, which often results in highly abundant genes being shown. The images also contain a grayscale for the combined abundance of all recorded transcripts (white=high, black=low). Depending on the distribution and abundance of the colored genes, the grayscale may not be visible.

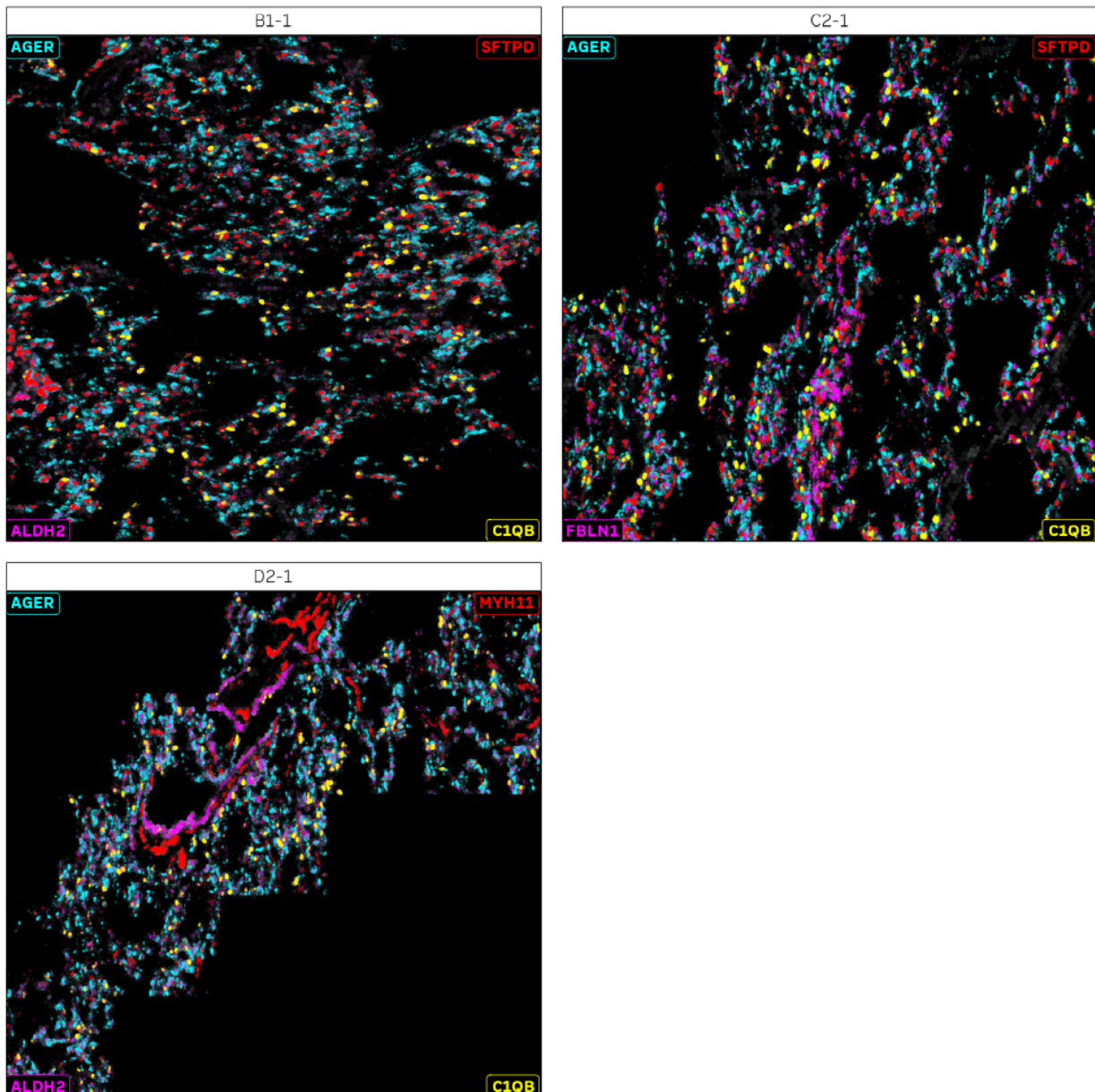


Figure 1: ROI-specific, high abundance, spatially distributed genes.

2.2 // Moderate abundance spatially distributed genes

Figure 2 highlights four selected genes in each ROI. In order to provide a clearer image, the colors of these genes may differ in subsequent ROIs. The genes are selected their distribution between areas of the respective ROI, which often results in moderately abundant genes being shown. The images also contain a grayscale for the combined abundance of all recorded transcripts (white=high, black=low). Depending on the distribution and abundance of the colored genes, the grayscale may not be visible.

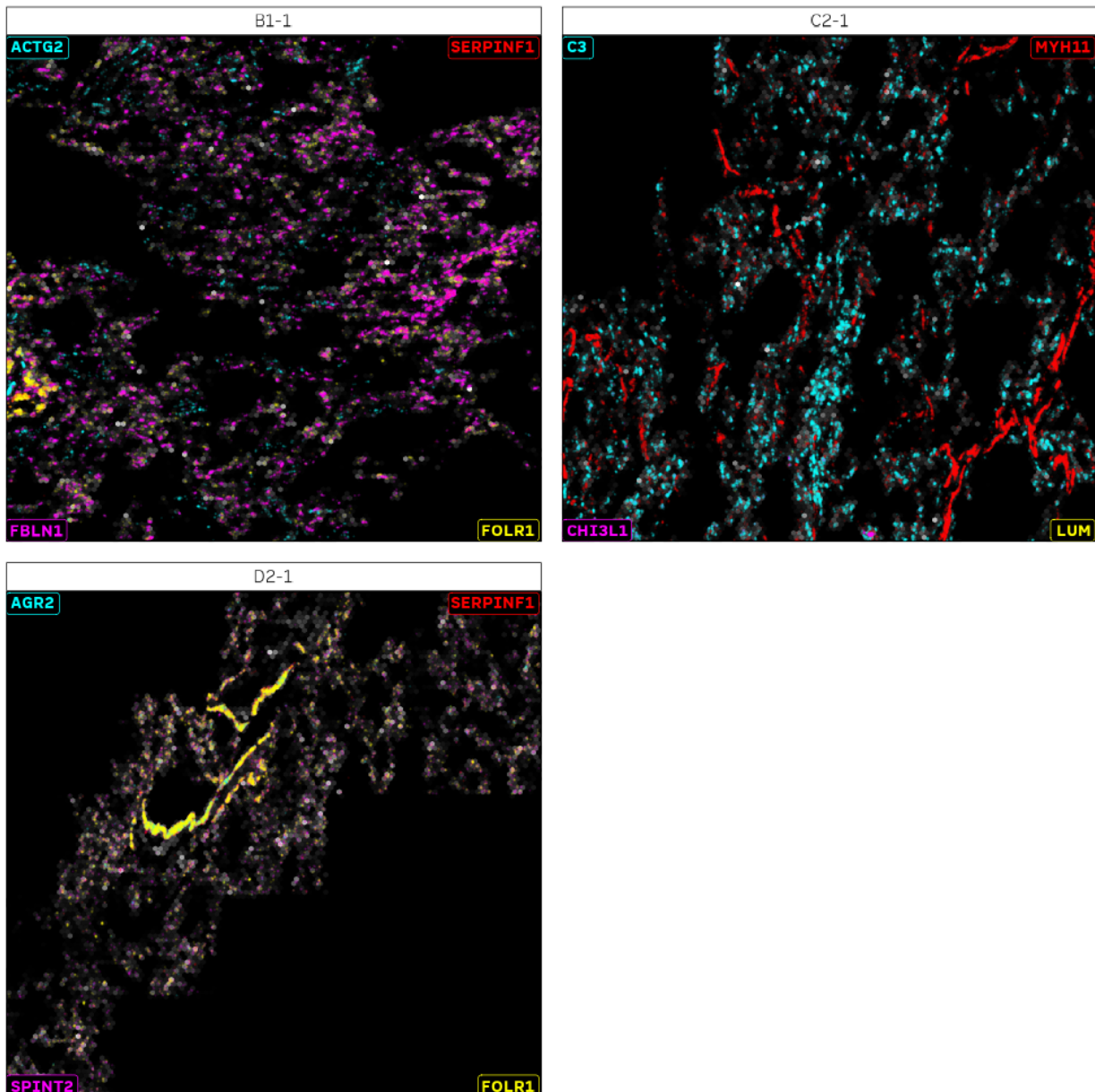


Figure 2: ROI-specific, moderate abundance, spatially distributed genes.

2.3 // Combination of ROI-specific genes

Figure 3 shows all ROIs with several genes from Figures 1 and 2. ROIs can directly be compared, however identifying genes can be challenging since the number of colors might be overwhelming and genes may overshadow each other. The images contain a grayscale for the abundance of all recorded transcripts combined (white=high, black=low). Depending on the distribution and abundance of the colored genes, the grayscale may not be visible.

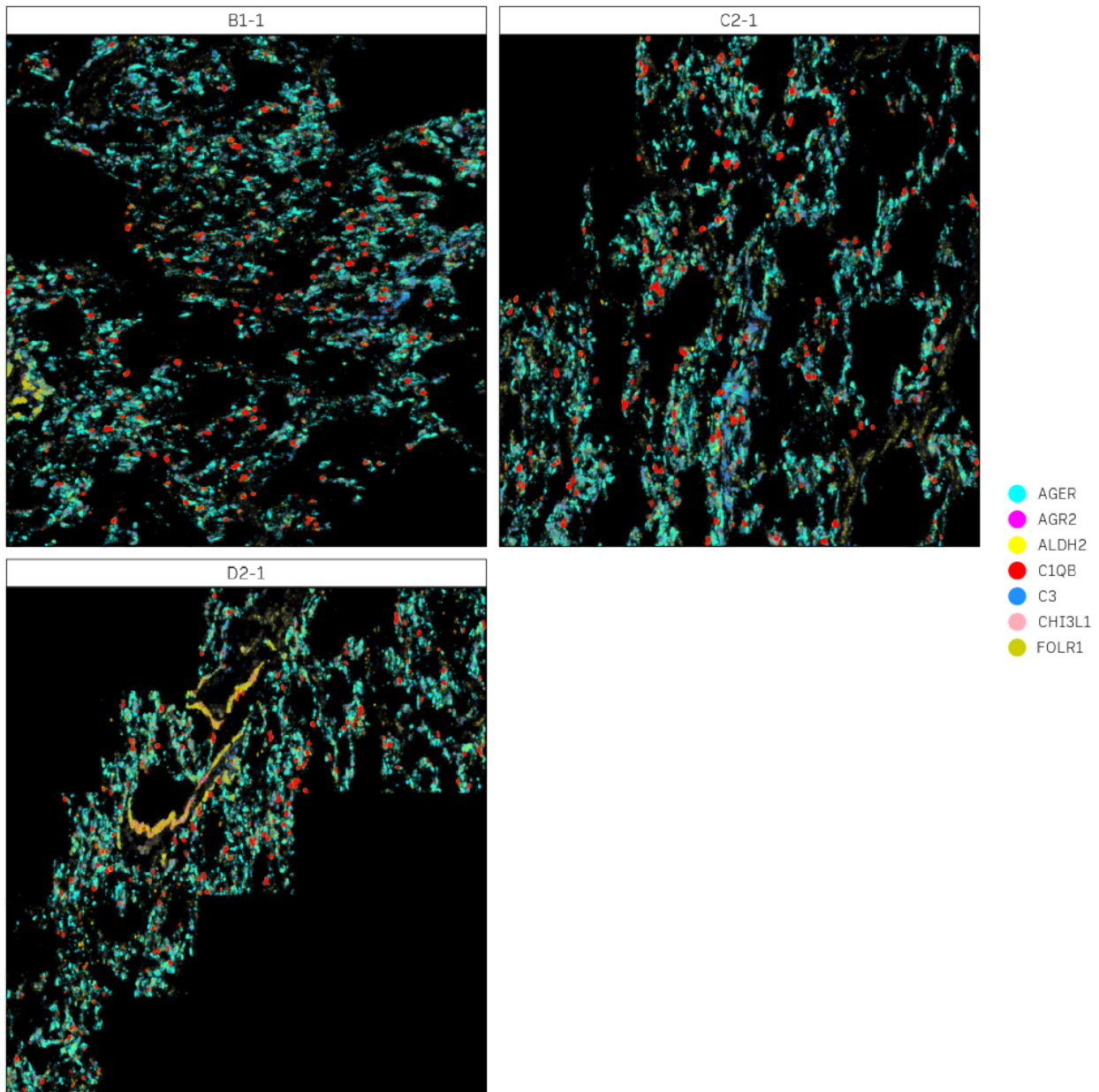


Figure 3: Combined highly- and moderately-abundant genes for all ROIs.

2.4 // Clusters within ROIs

Each ROI is divided into a maximum of 32 x 32 tiles. These tiles are then clustered based on the abundance of all genes inside each tile.

2.4.1 Tile clustering

Figure 4 visualizes tiles as rectangles. Colors indicate different clusters, while black regions contain only few transcripts and are omitted from the analysis.

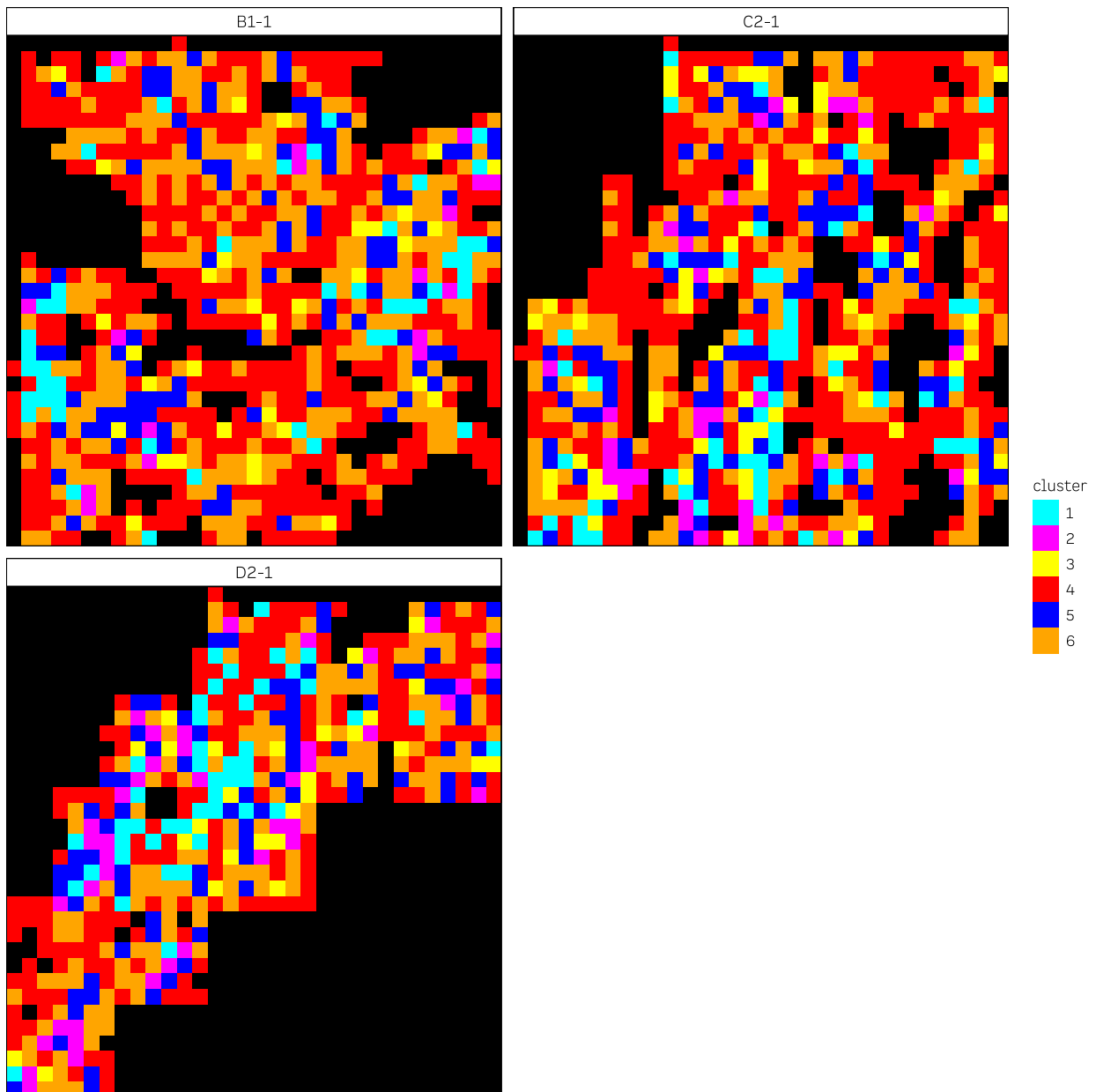


Figure 4: Tile clustering by gene abundance.

2.4.2 Gene patterns in clusters

The heatmaps in Figure 5 represent the mean abundance of genes for each cluster inside a given ROI. Genes were selected based on their standard deviations between clusters and their highest abundance in any given cluster.

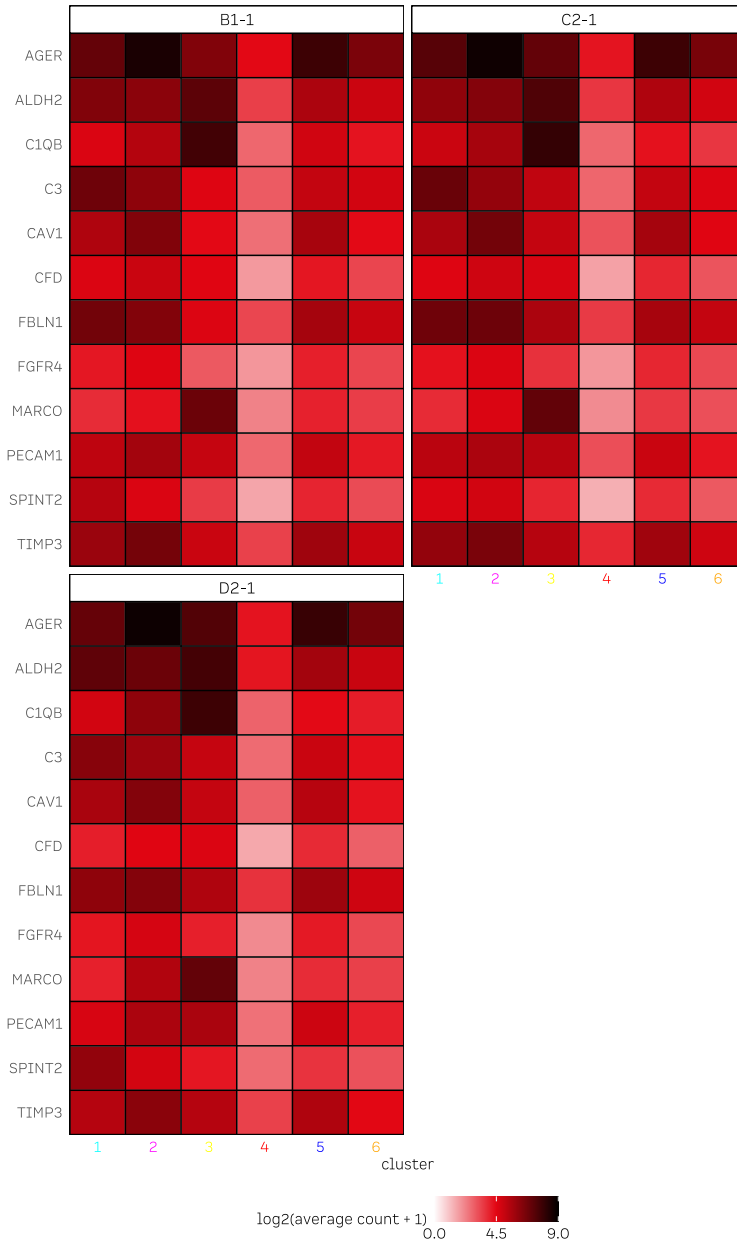


Figure 5: Heatmap showing cluster-specific abundance of genes for each ROI.

3.1 // Total counts and false positive rates

The count of a ROI is the number of transcript molecules identified within it. Figure 6 shows the total combined number of counts for all targeted genes in each ROI. The exact numbers divided by 1000 are noted to the right of each bar. The percentage of estimated false positives in relation to the total count is written in the box on the left side of each ROI bar.

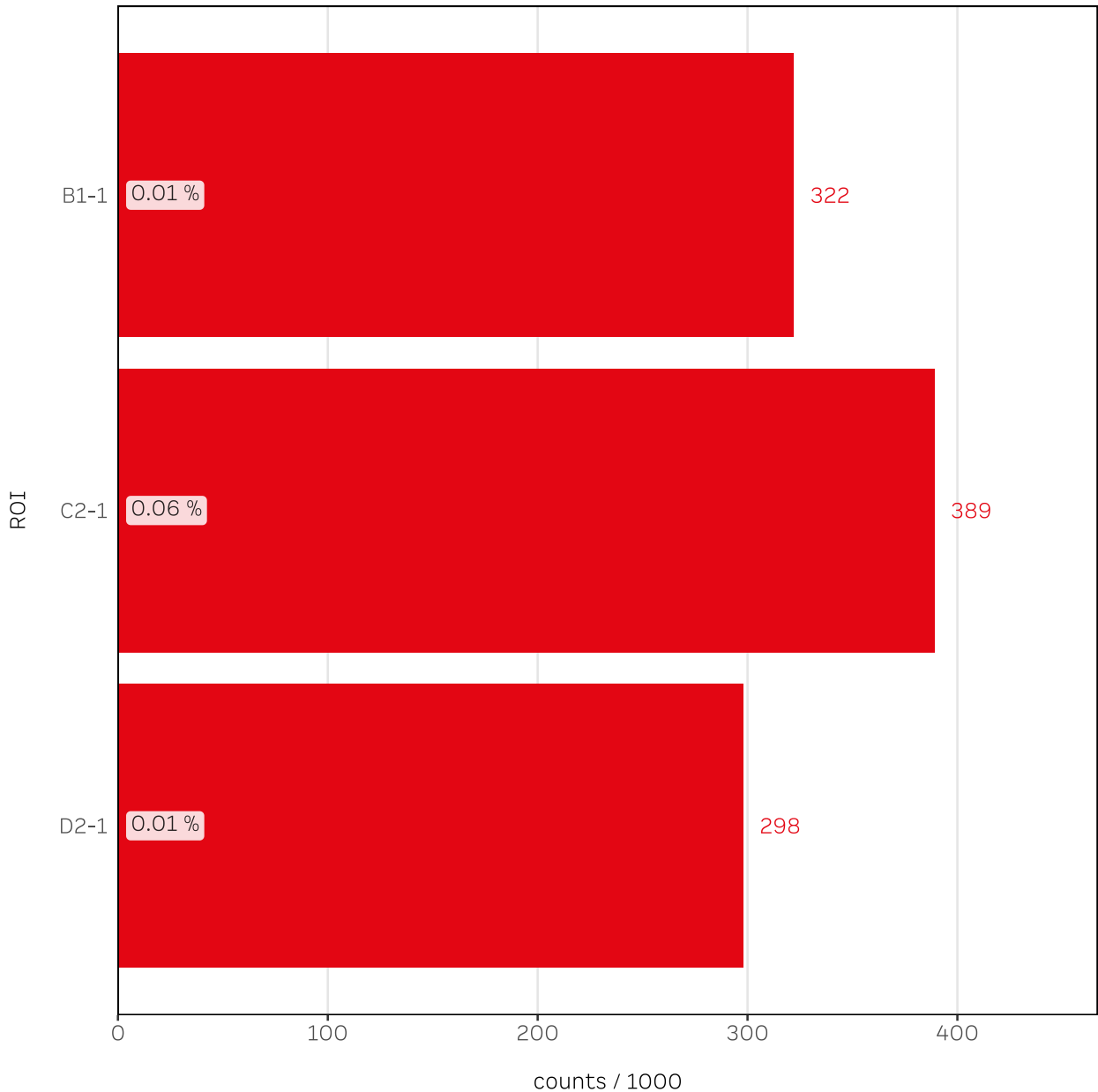


Figure 6: Counts from all genes combined.

3.2 // Individual gene counts

The top left heatmap in Figure 7 represents the transcript count, where each row represents a gene. The boxplot summarizes the genes (red) and false positive controls (FPC, black). For each box, the white middle bar indicates the median, while the box ranges from the 25th to the 75th percentile. Based on the transcript count, the Pearson correlation in the lower panel indicates similarity of ROIs to each other.

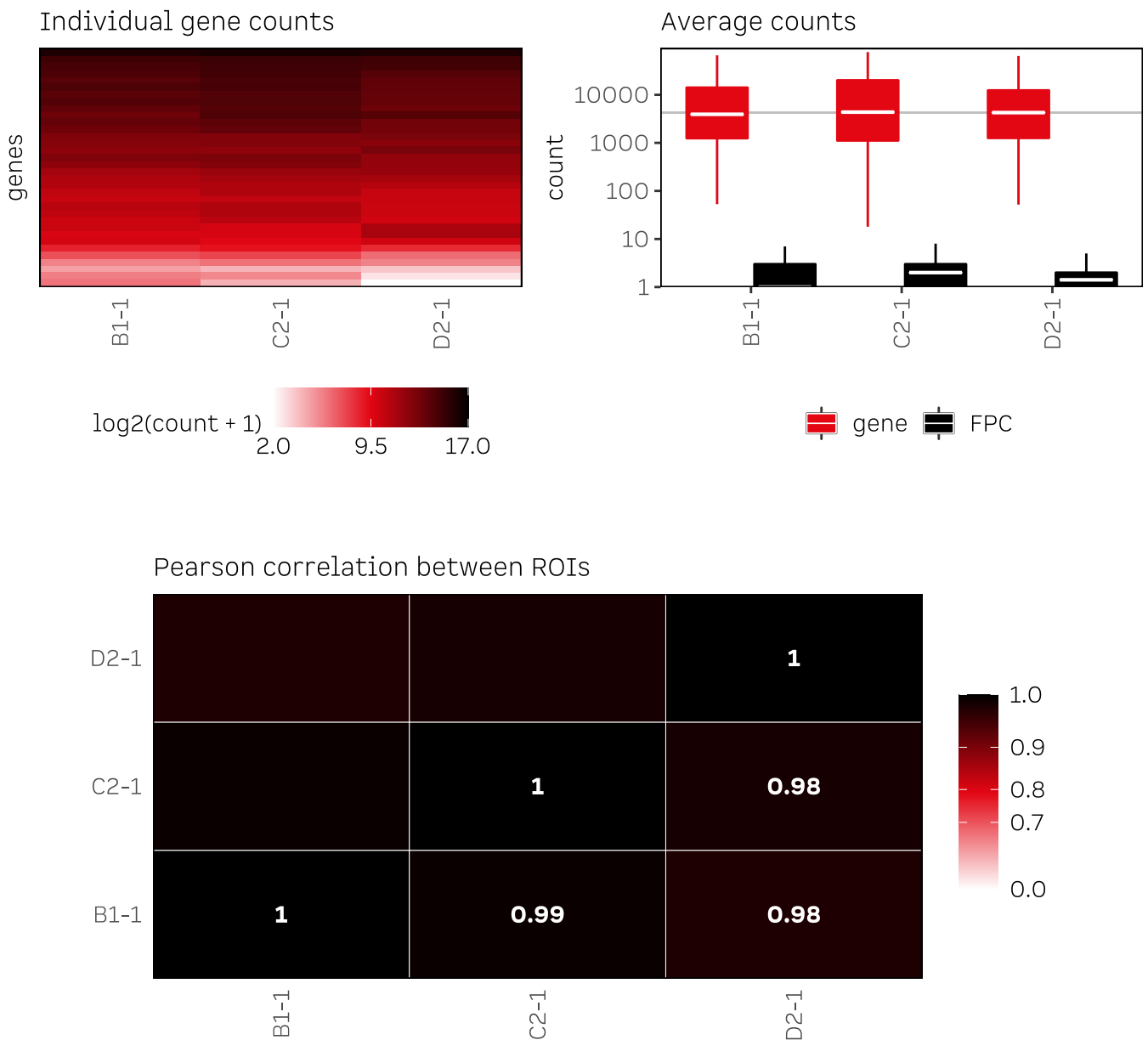


Figure 7: Gene abundancies for each ROI and Pearson correlations between ROIs.

4.1 // Quality cutoffs

Genes with few signals should spark some caution because much of their signal may be due to noise. Locally expressed genes, however, may have few transcripts that are concentrated in distinct regions. The maximum local count is the highest count among subregions of the ROI and is used to call out problematic genes without falsely including locally abundant ones. In Figure 8, genes are colored red if they fall below the threshold, represented by the vertical dashed line and based on the false positive rate for that ROI. The horizontal dashed line depicts a threshold for the quality score, a measure for each recorded transcript molecule. Genes below this threshold are colored blue. Genes marked blue, red, or purple (crossing both thresholds) should be handled with caution. They are listed with their number in Figure 9.

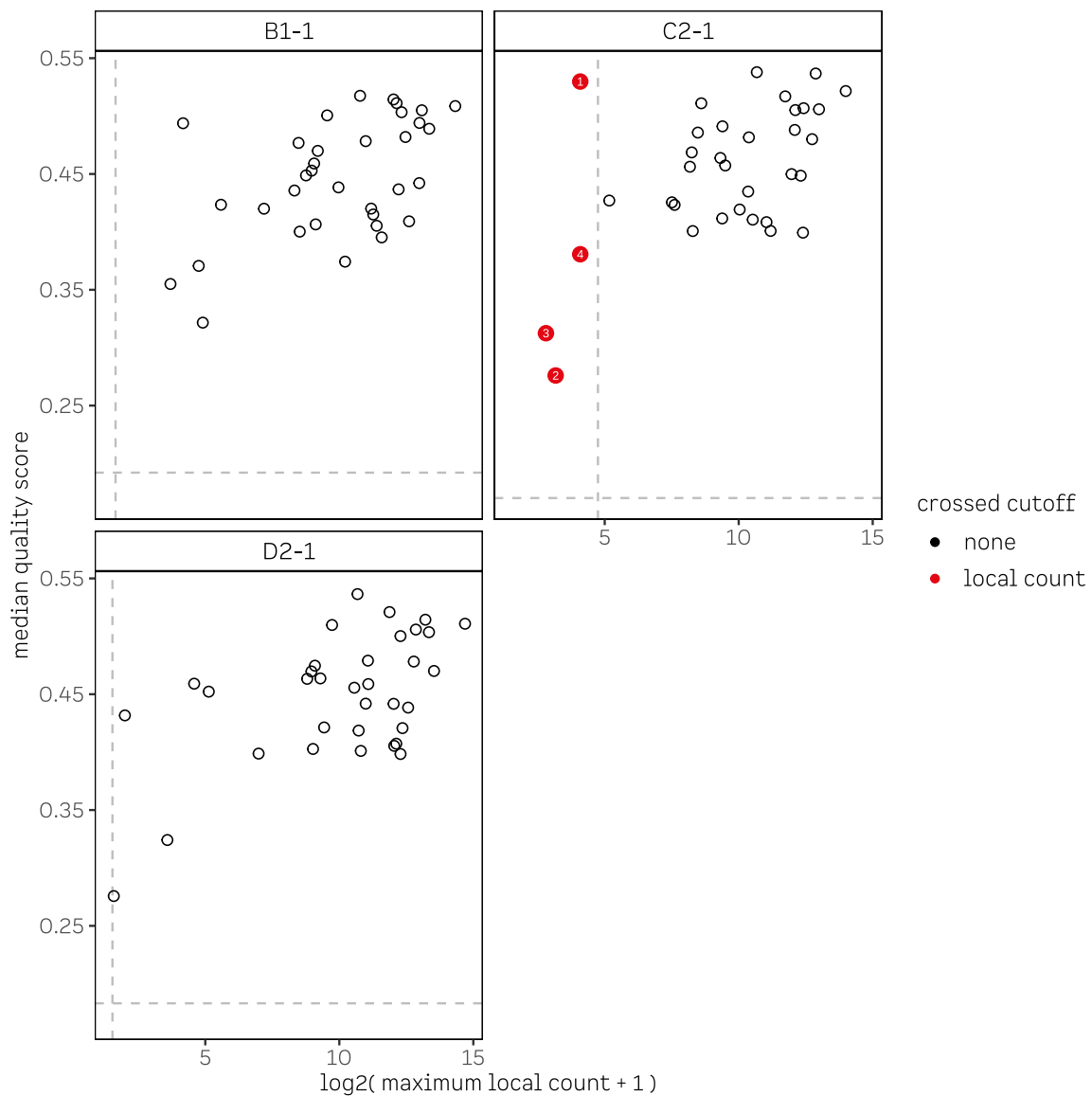


Figure 8: Transcript quality and local abundance.

4.2 // List of genes below cutoffs

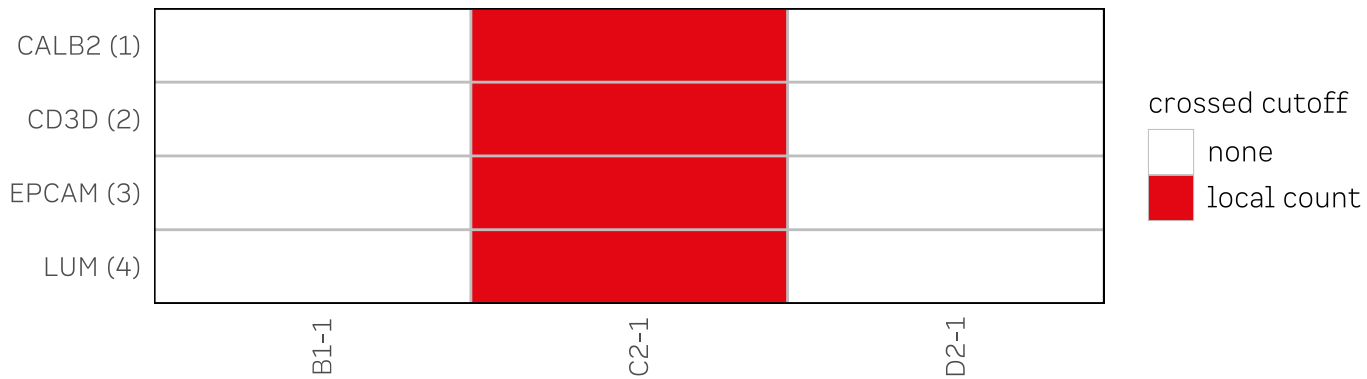


Figure 9: Genes below cutoffs.

This report is a compilation of your data quality and provides insights into prominent markers. You should have received DAPI images and transcript tables, only the latter of which has been used for this report. In the images, each pixel has a width and height of 138 nm (x- and y-axis in 2-dimensional space). We have provided you with a selection of tools to use when analyzing your data.

5.1 // ReCognize™ - a free online tool

The ReCognize tool enables easy visualization and analysis of your data. No prior experience is required. We recommend visiting this first when accessing your data. You can access ReCognize via our customer portal at <https://my.resolvebiosciences.com/>. It enables you to:

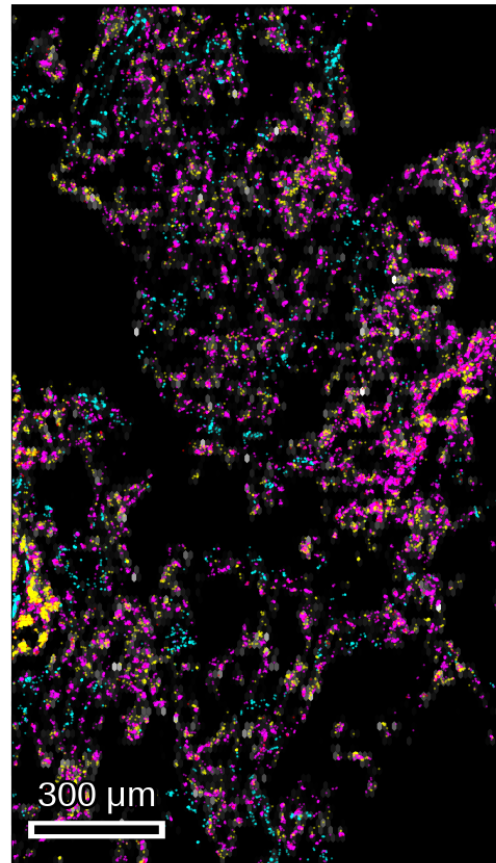
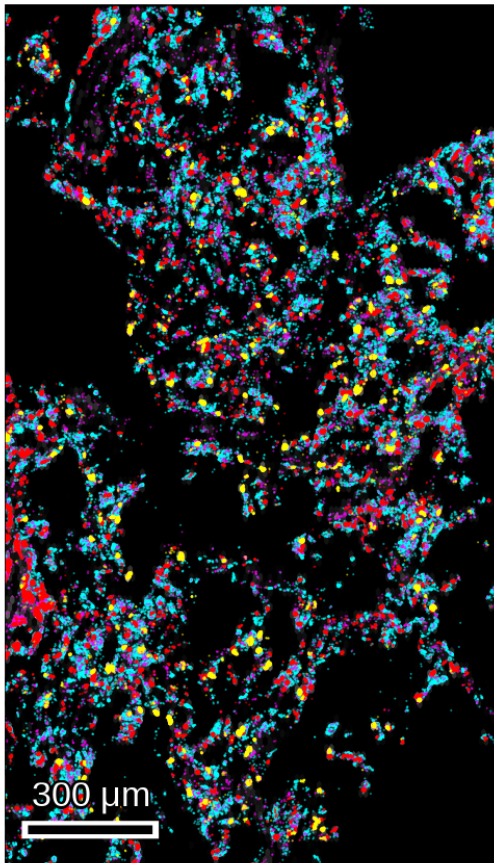
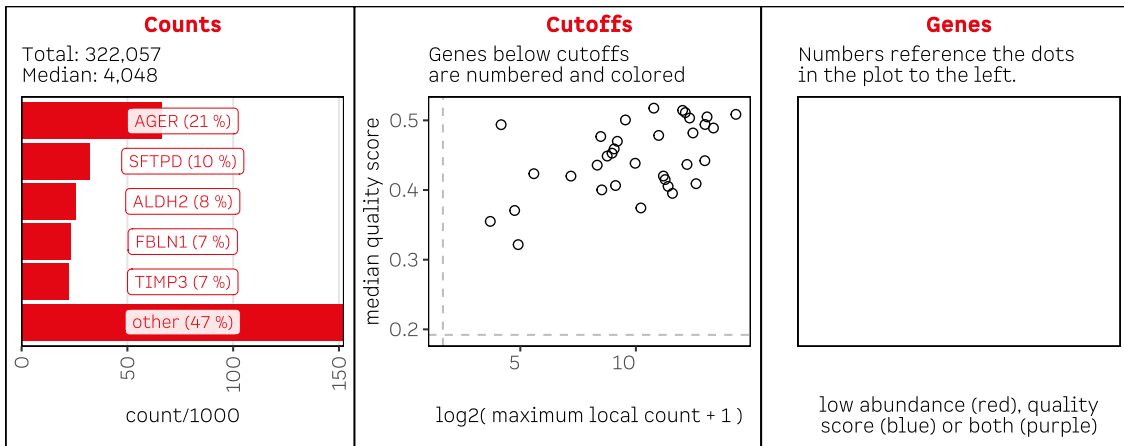
- look at your data in 3-D
- colorize transcripts
- look at multiple metrics
- include regions of interest from ImageJ and
- cluster these regions based on transcript count and identify important genes

5.2 // Polylux™ - an ImageJ/Fiji plugin

This plugin provides greater flexibility and customizable analysis. Prior ImageJ experience is beneficial but not necessary. This tool was provided with your data package, along with instructions for use. You can access a training video [here](#). The tool has many features which are constantly improved to ensure you obtain the greatest insights from your data. The plugin provides, but is not limited to:

- a density map
- co-localization analysis
- heatmap and dendrogram generation
- the platform itself: ImageJ/Fiji comes with many features and enjoys an active community providing plugins for many purposes

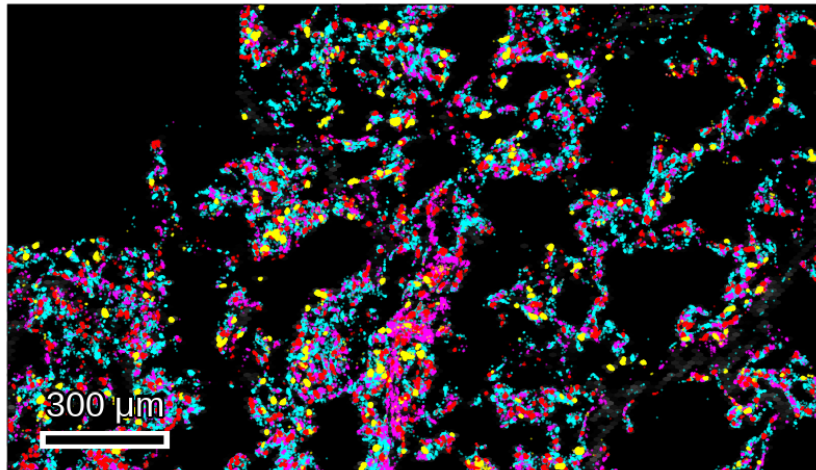
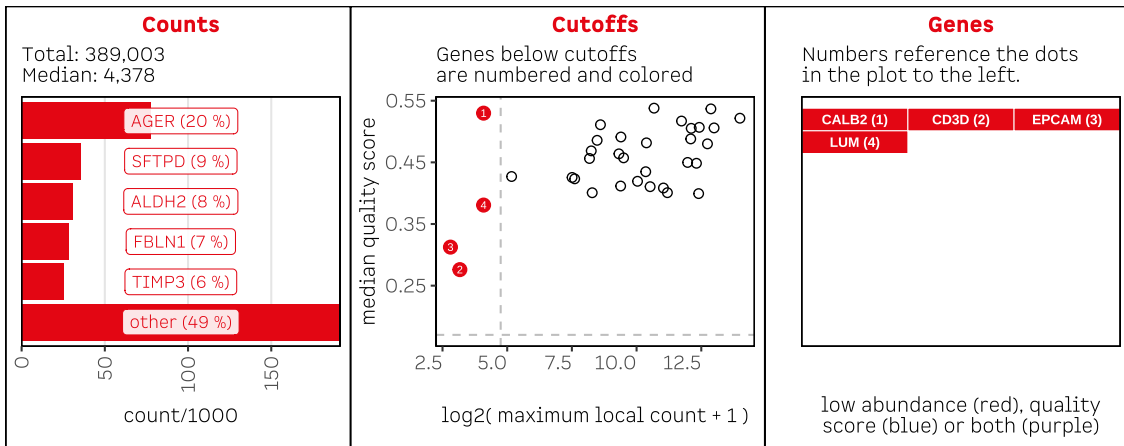
ROI summary: B1-1



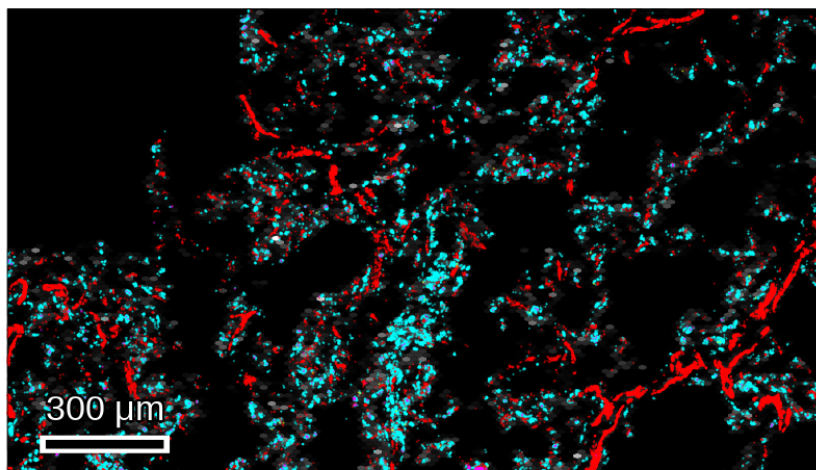
● AGER ● ALDH2 ● C1QB ● SFTPD

● ACTG2 ● FBLN1 ● FOLR1 ● SERPINF1

ROI summary: C2-1

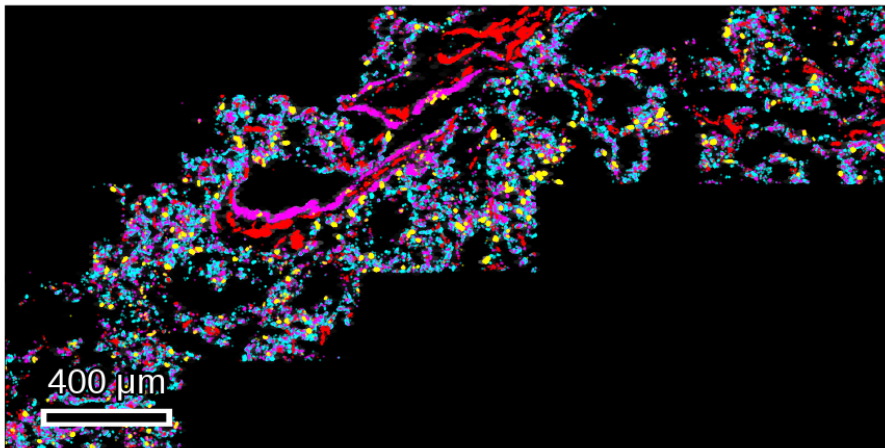
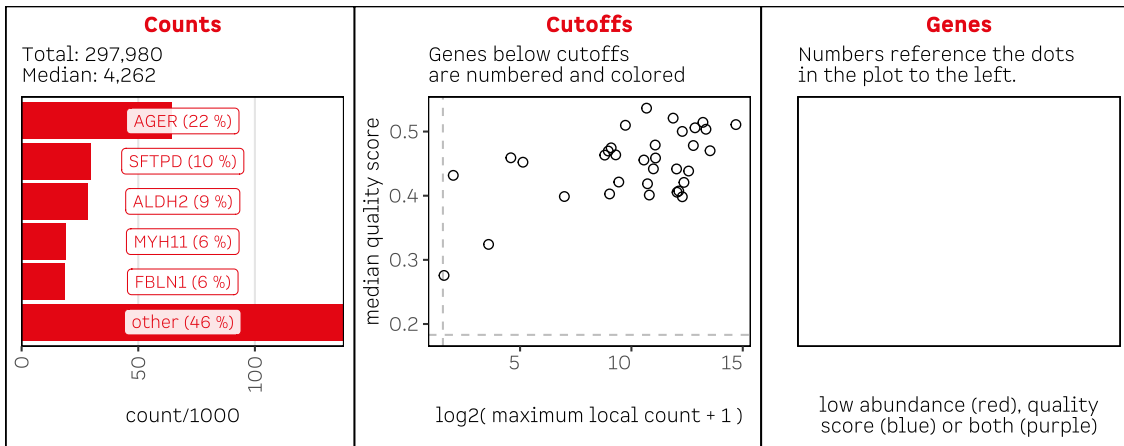


- AGER
- FBLN1
- C1QB
- SFTPD

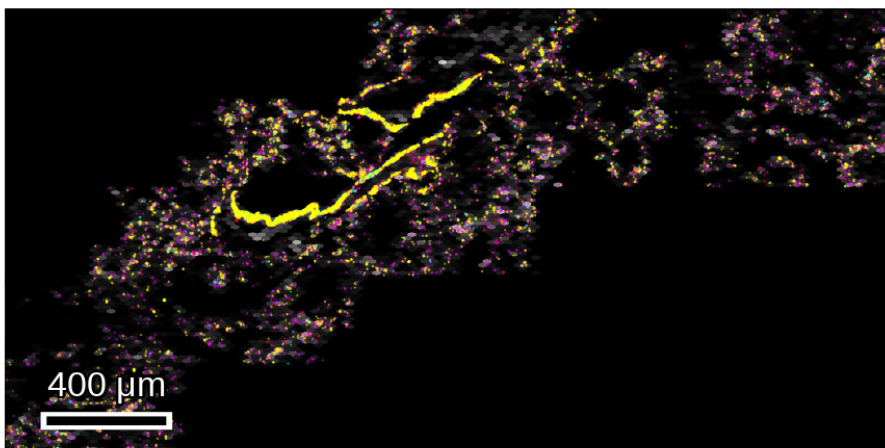


- C3
- CHI3L1
- LUM
- MYH11

ROI summary: D2-1



- AGER
- ALDH2
- C1QB
- MYH11



- AGR2
- SPINT2
- FOLR1
- SERPINF1

This report was produced via R (R Core Team (2021a)) and Pandoc (John MacFarlane (2006)). Several R packages were used to aid in PDF creation, data handling, calculation and visualization. The following references list all used packages, excluding those serving as their dependencies. Packages for PDF creation include rmarkdown (Allaire et al. (2021), Xie, Allaire, and Golemund (2018), Xie, Dervieux, and Riederer (2020)), knitr (Xie (2022), Xie (2015), Xie (2014)) and bookdown (Xie (2021), Xie (2016)). Data was handled with data.table (Dowle and Srinivasan (2021)), dplyr (Wickham et al. (2021)), scales (Wickham and Seidel (2020)), reshape2 (Wickham (2007)), stringr (Wickham (2019)), XML (Temple Lang (2021)), reldist (Handcock and Morris (1999), Handcock (2016)), Rfast (Papadakis et al. (2021)), bigmemory (Kane, Emerson, and Weston (2013)), biganalytics (Emerson and Kane (2020)). Visualization was performed using ggplot2 (Wickham (2016)), ggpubr (Kassambara (2020)), patchwork (Pedersen (2020)), shadowtext (Yu (2021)), magick (Ooms (2021)), showtext (Qiu and See file AUTHORS for details. (2021)), grid (R Core Team (2021b)), ggtext (Wilke (2020)).

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resolve
biosciences

Resolve BioSciences GmbH
Creative Campus Monheim, Gebäude A03

• Alfred-Nobel-Str. 10,
40789 Monheim am Rhein, Germany

• (+49) 2173 2975-200
info@resolve-biosciences.com