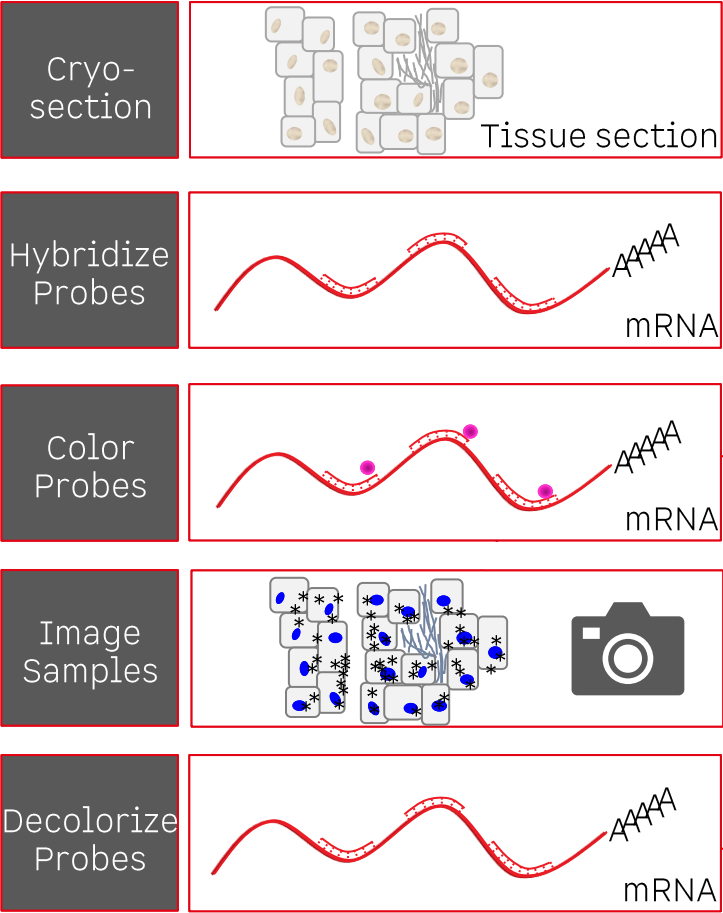




Molecular Cartography™ Results

31 August 2021

Workflow



Cryosectioning

Hybridization of transcript-specific probes

Colouring of probes enabled by new technology

Imaging of sections

Decolorization of probes

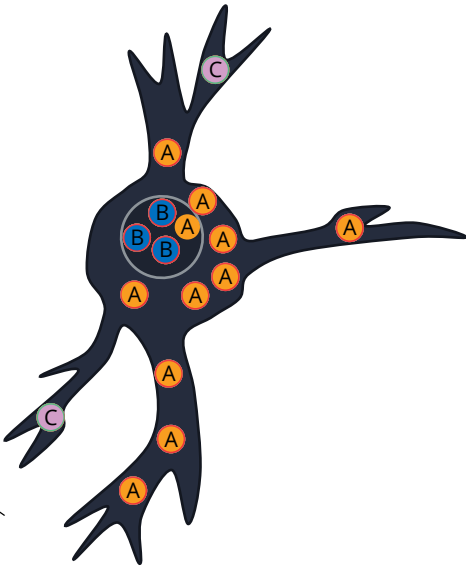
Code:

	round 1	round 2	round 3	round 4	round 5	round n
Transcript A	●	●	●	●	●	●
Transcript B	●	●	●	●	●	●
Transcript C	●	●	●	●	●	●

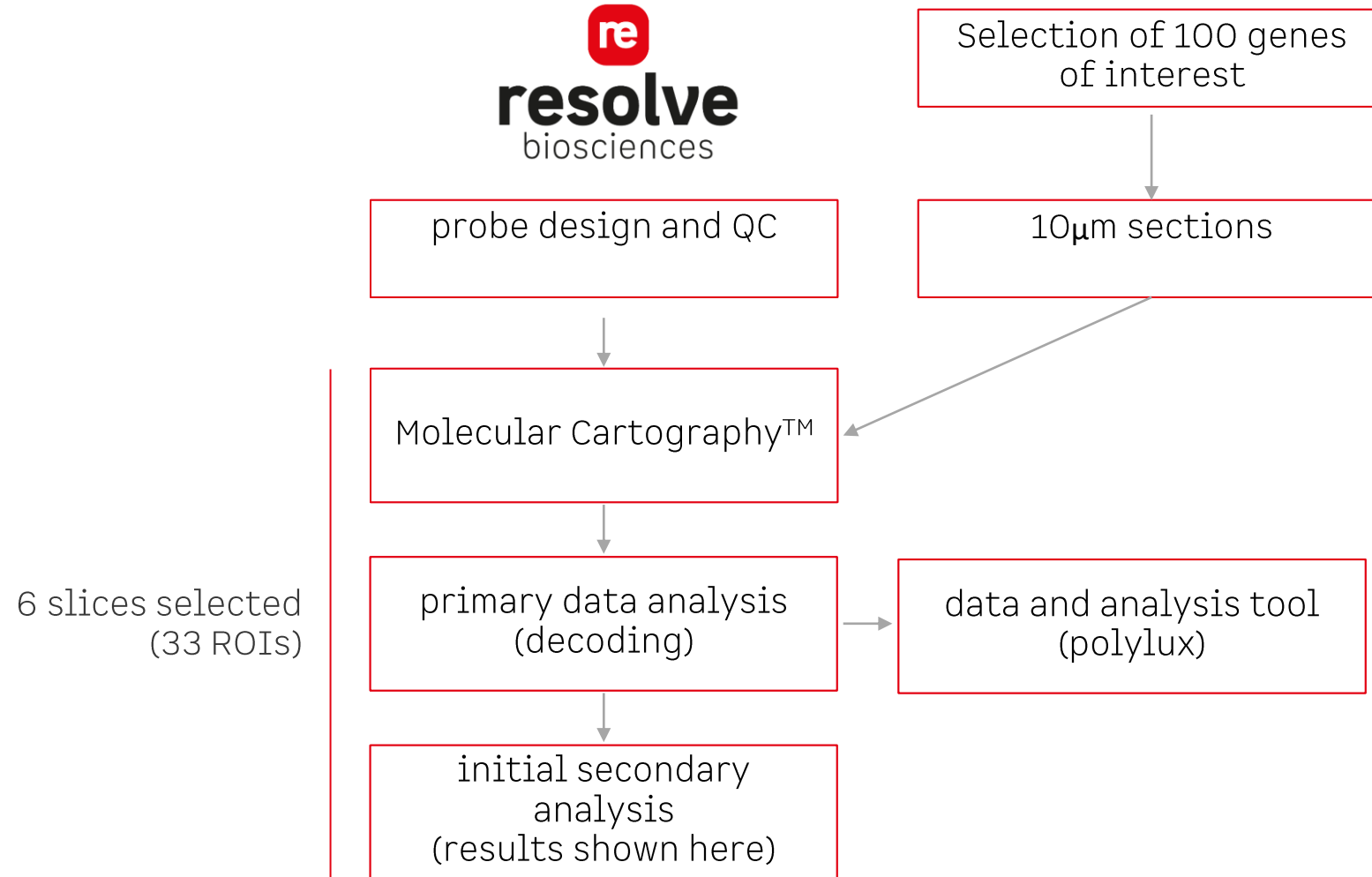
Transcript A

Transcript B

Transcript C

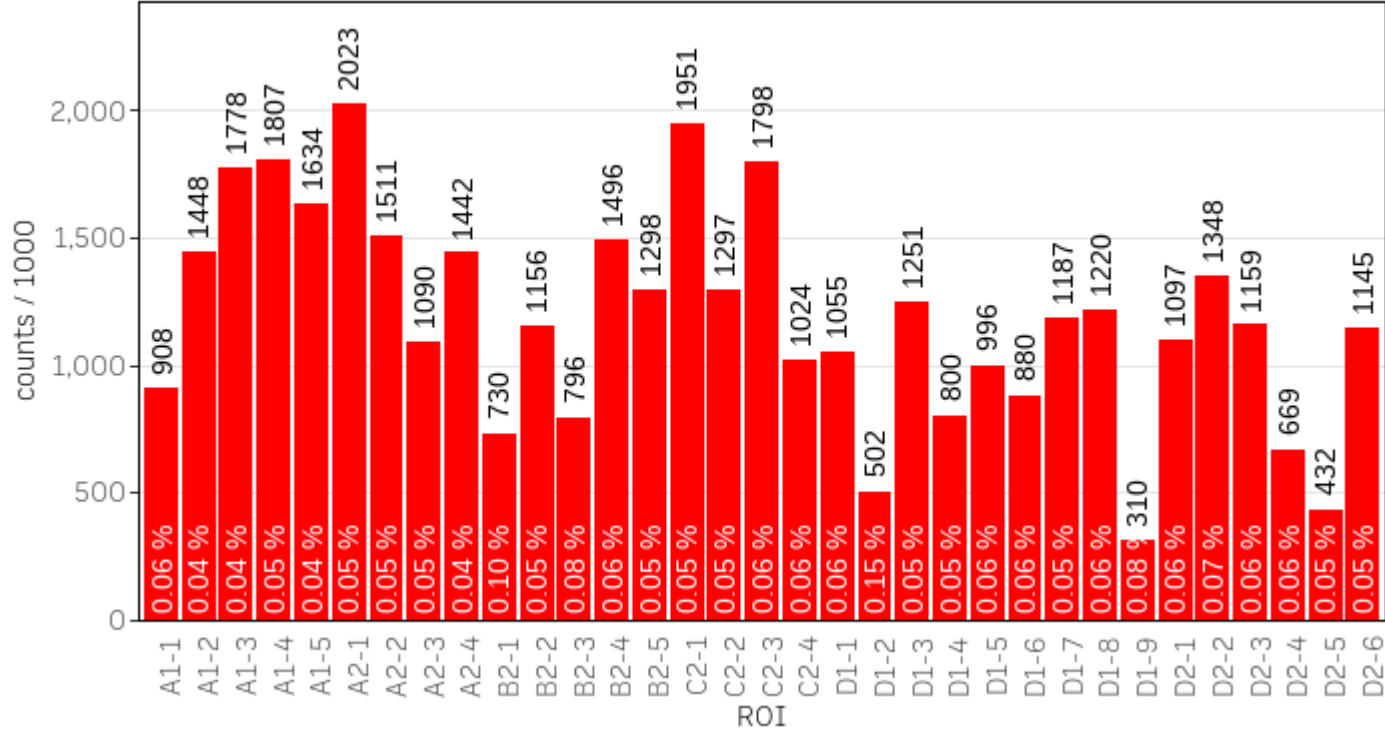


Project Structure

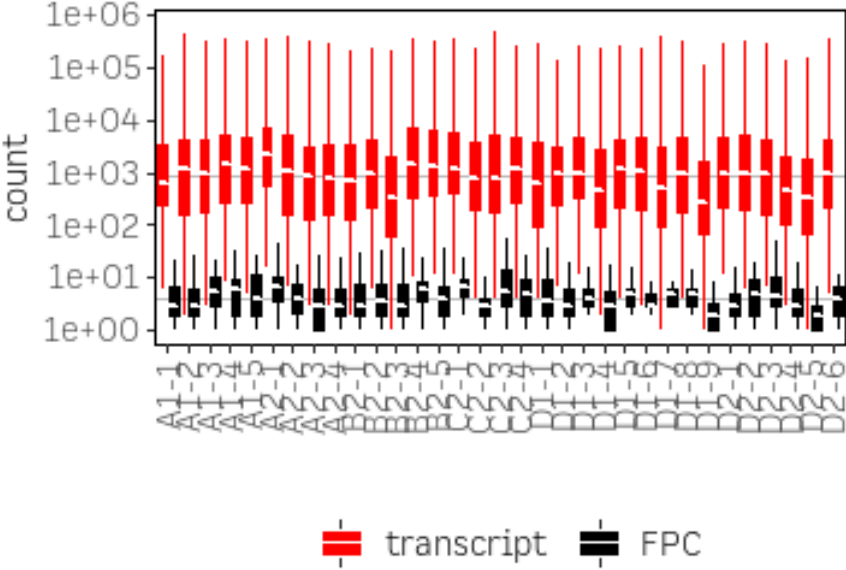


Overview

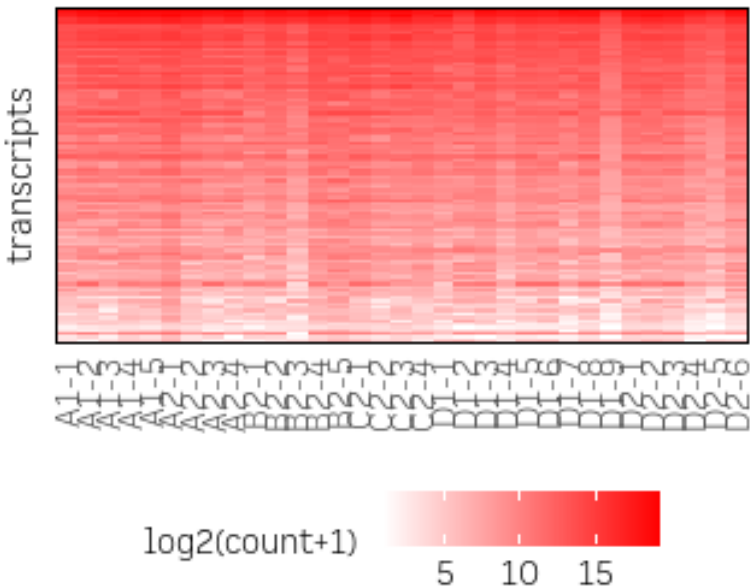
Combined transcript counts



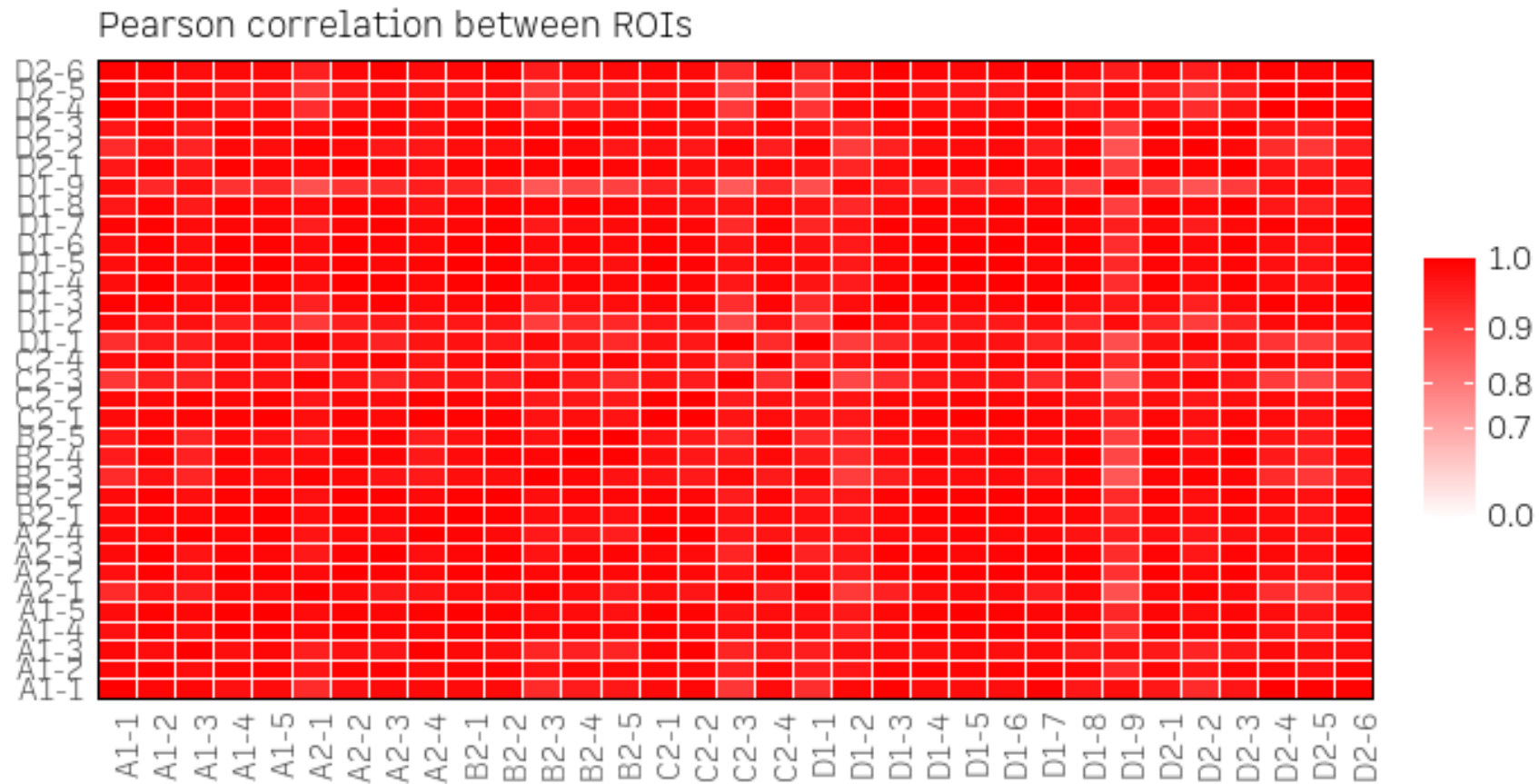
Average counts



Individual transcript counts



Good correlation between total counts across samples

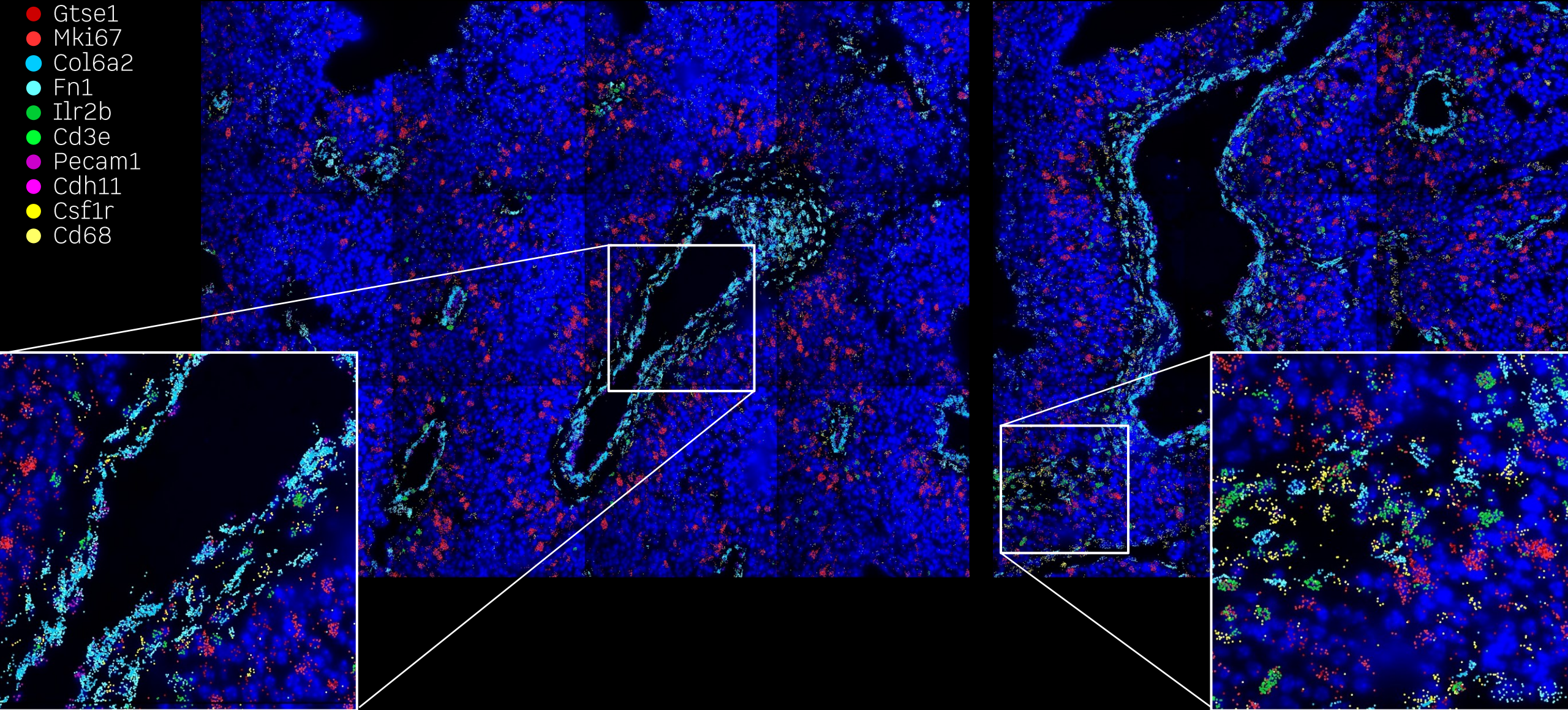


Example for transcripts with a distinct spatial distribution

A1-3

D2-6

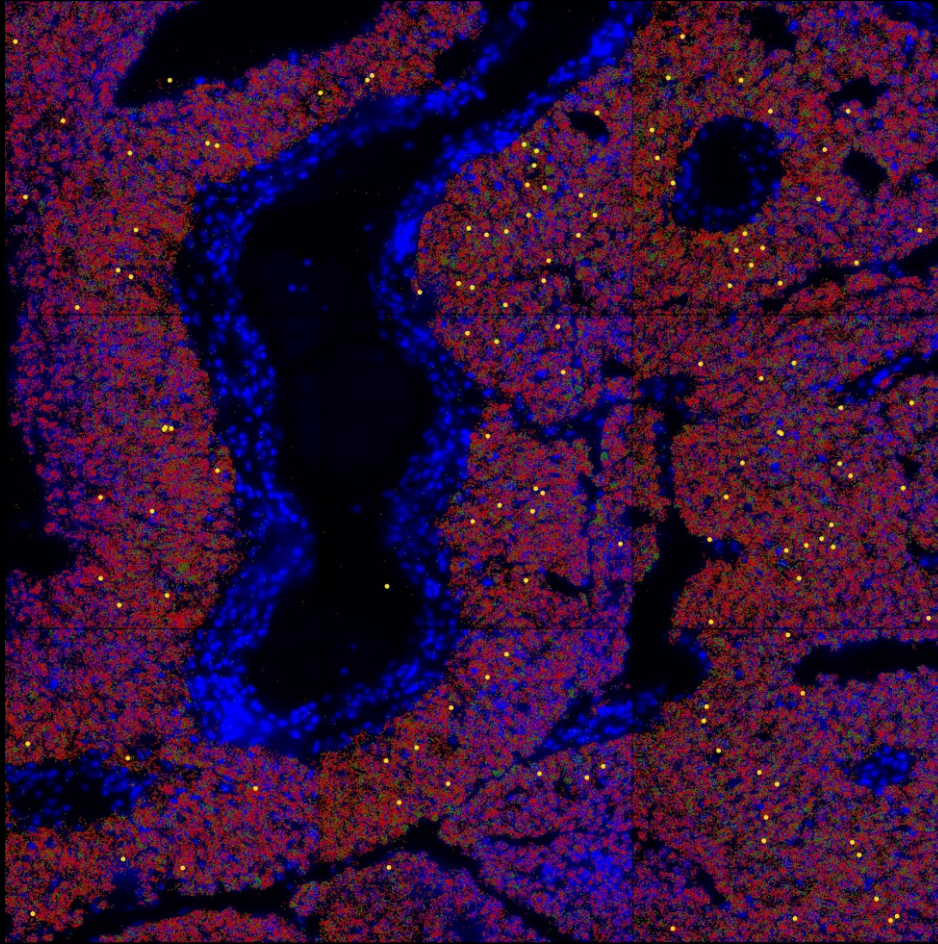
- Gtse1
- Mki67
- Col6a2
- Fn1
- Ilr2b
- Cd3e
- Pecam1
- Cdh11
- Csf1r
- Cd68



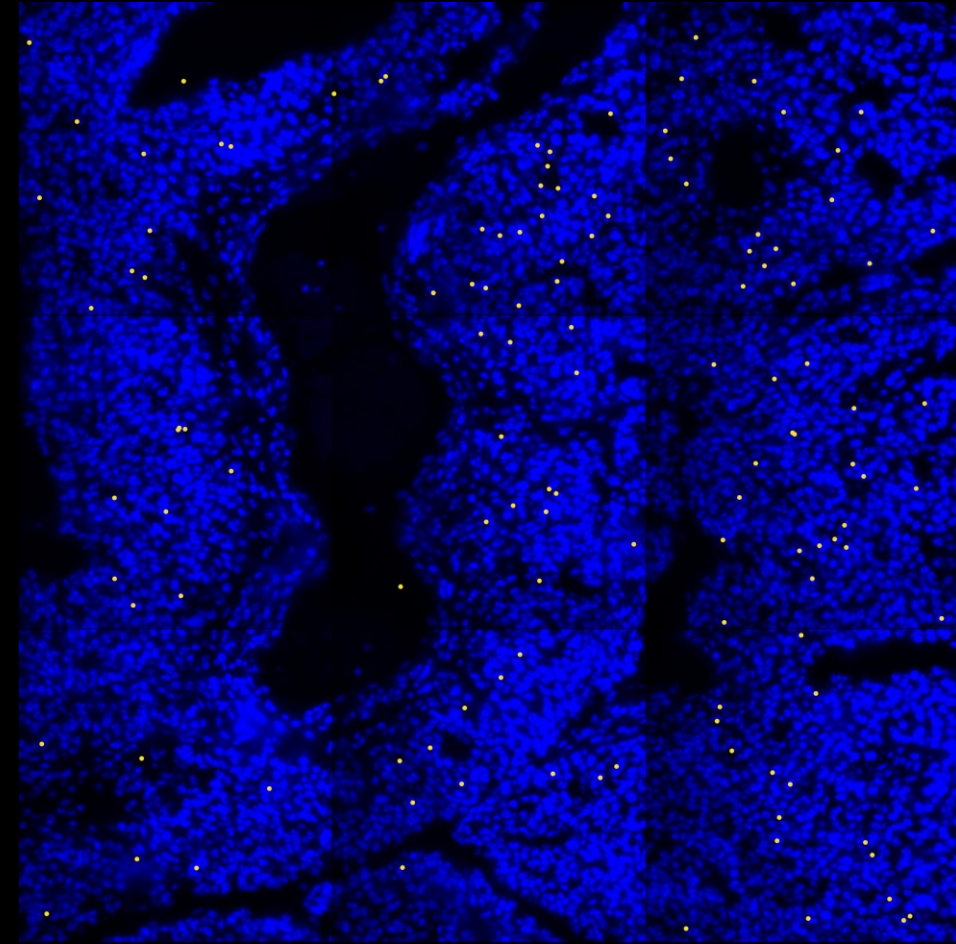
Little NRAS detected

● NRAS
● Mitf
● Sox10

D2-6 (NRAS, Mitf, Sox10)

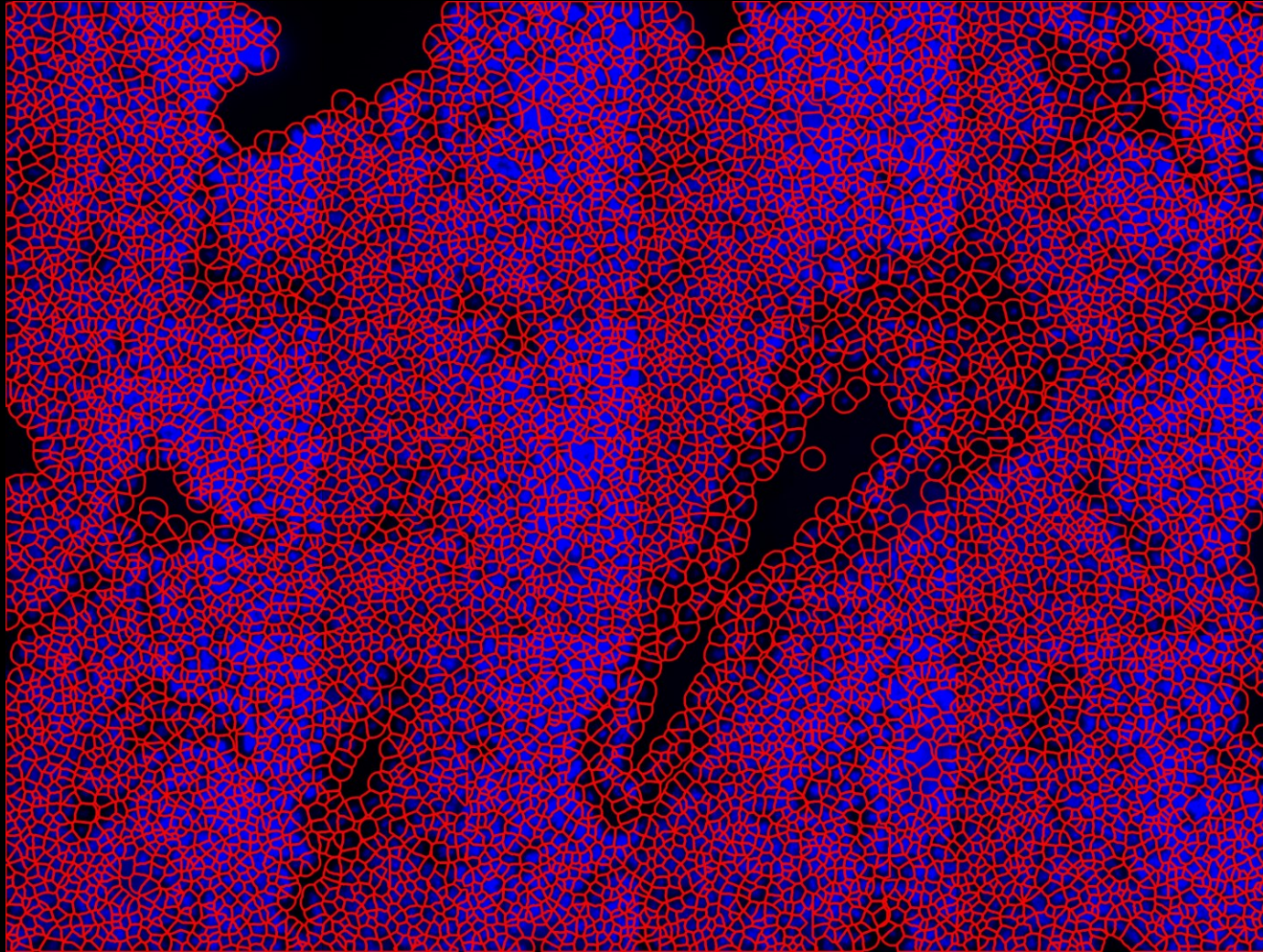


D2-6 (NRAS only)



Example for transcripts with a distinct spatial distribution

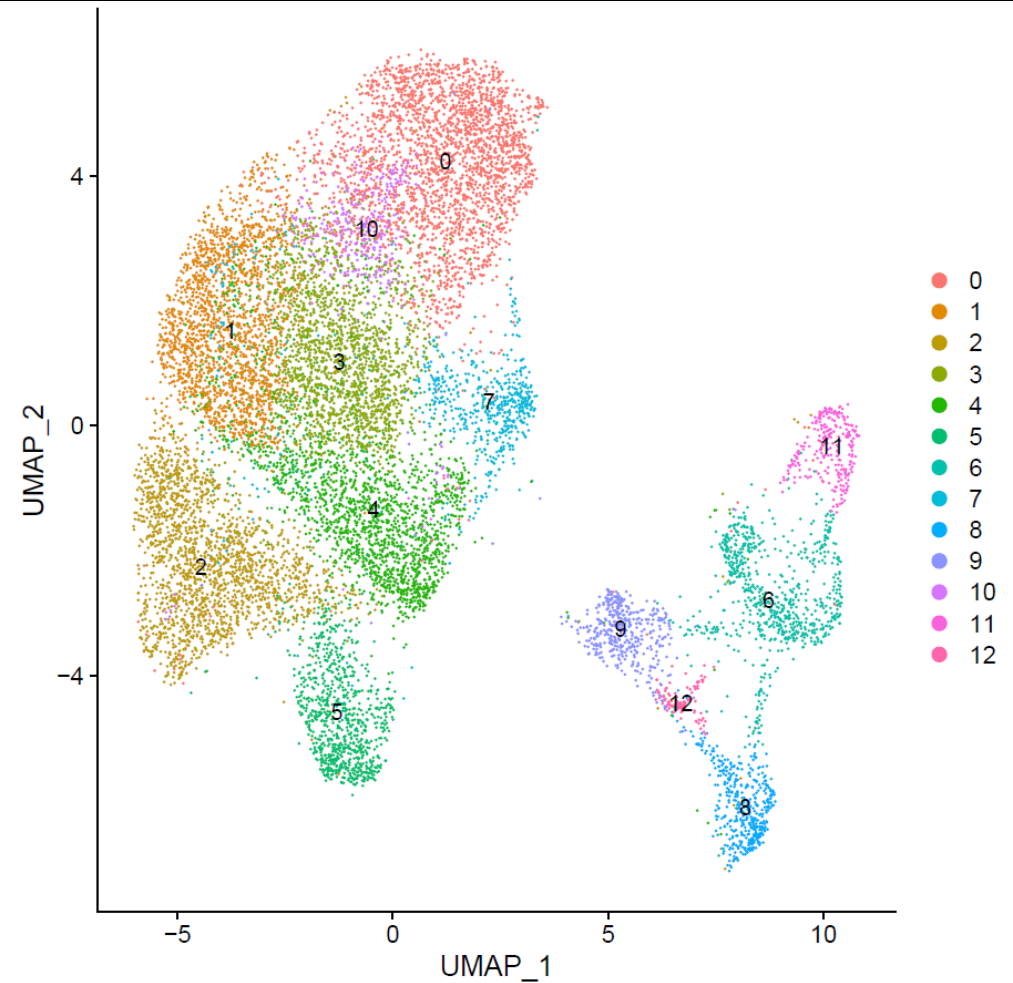
Single molecule signals per cell (ImageJ ROI) can be counted in the polylux tool and used for cell identification or clustering.



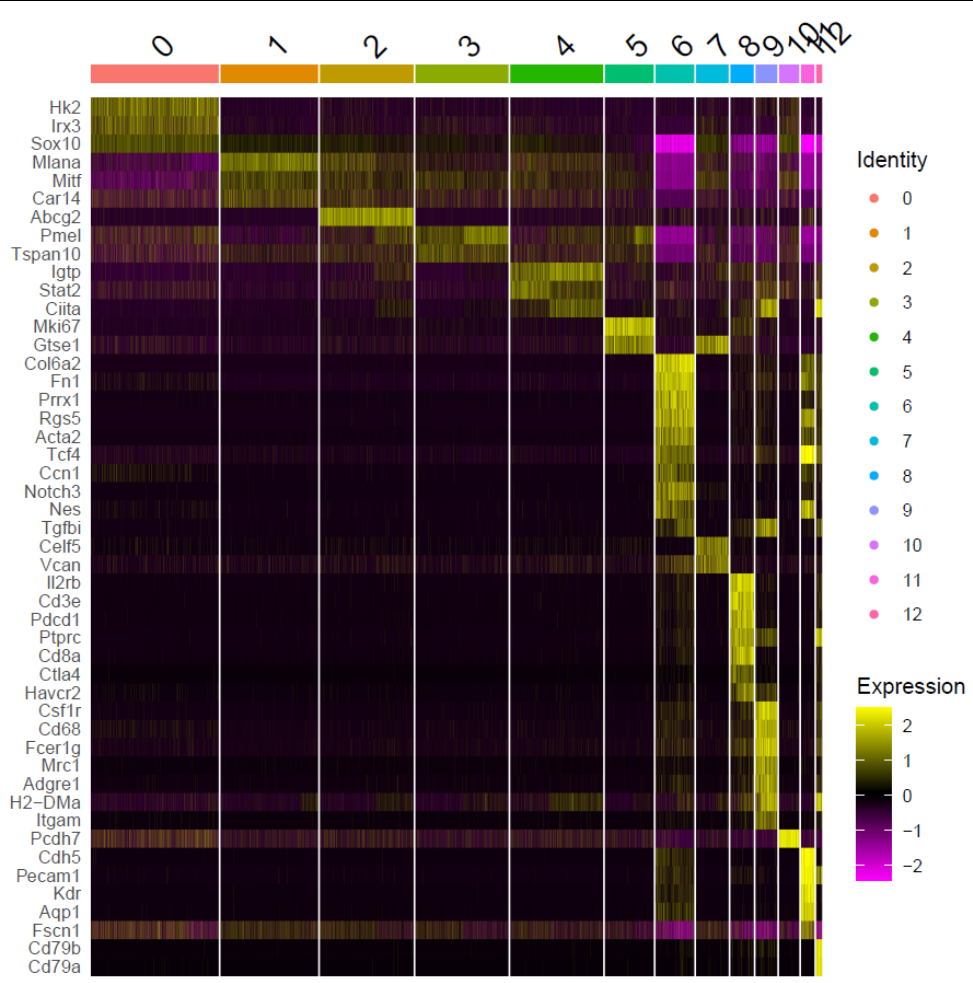
Segmentation performed with QuPath (Bankhead *et al.* Scientific Reports 2017) and DAPI signals expanded by 8 μm .

An example for cell clustering using Seurat (Stuart, Buttlar *et al.* Cell 2019)

UMAP plot of cells clustered by log-normalized transcript counts

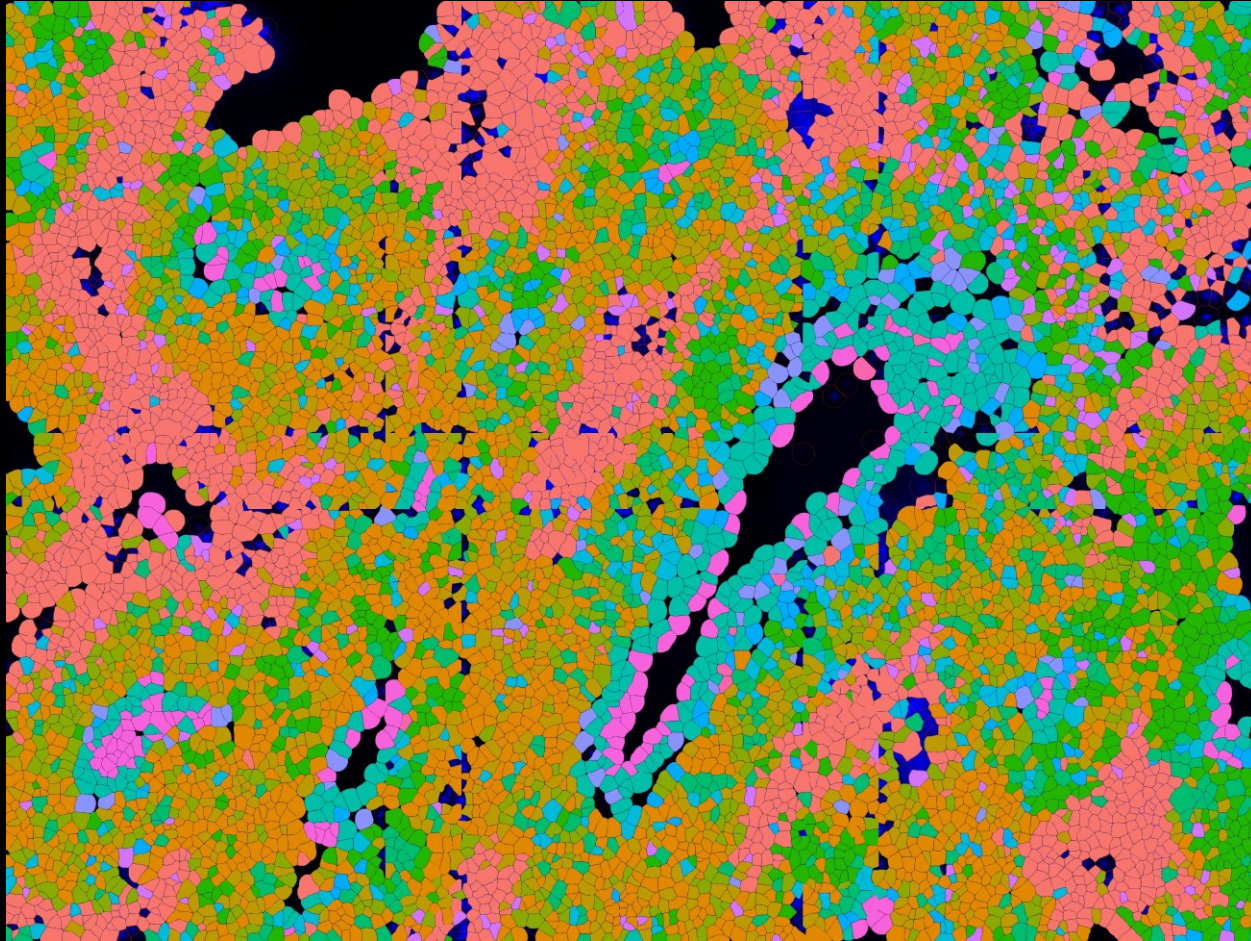


Most highly enriched markers in a cluster relative to all other cells.

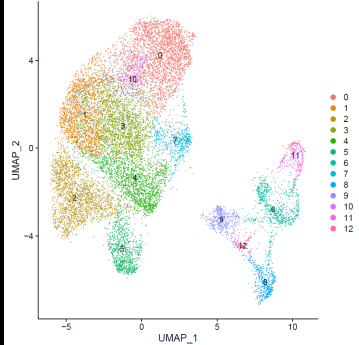
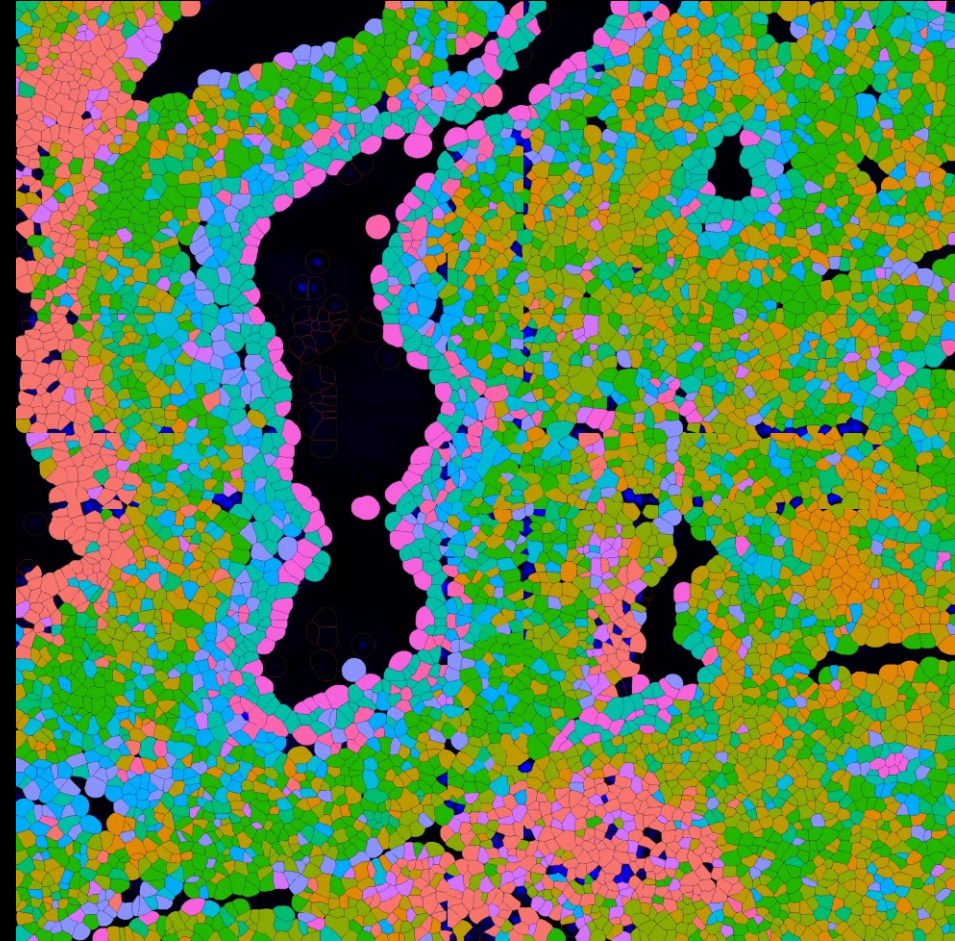


An example for cell clustering using Seurat

A1-3

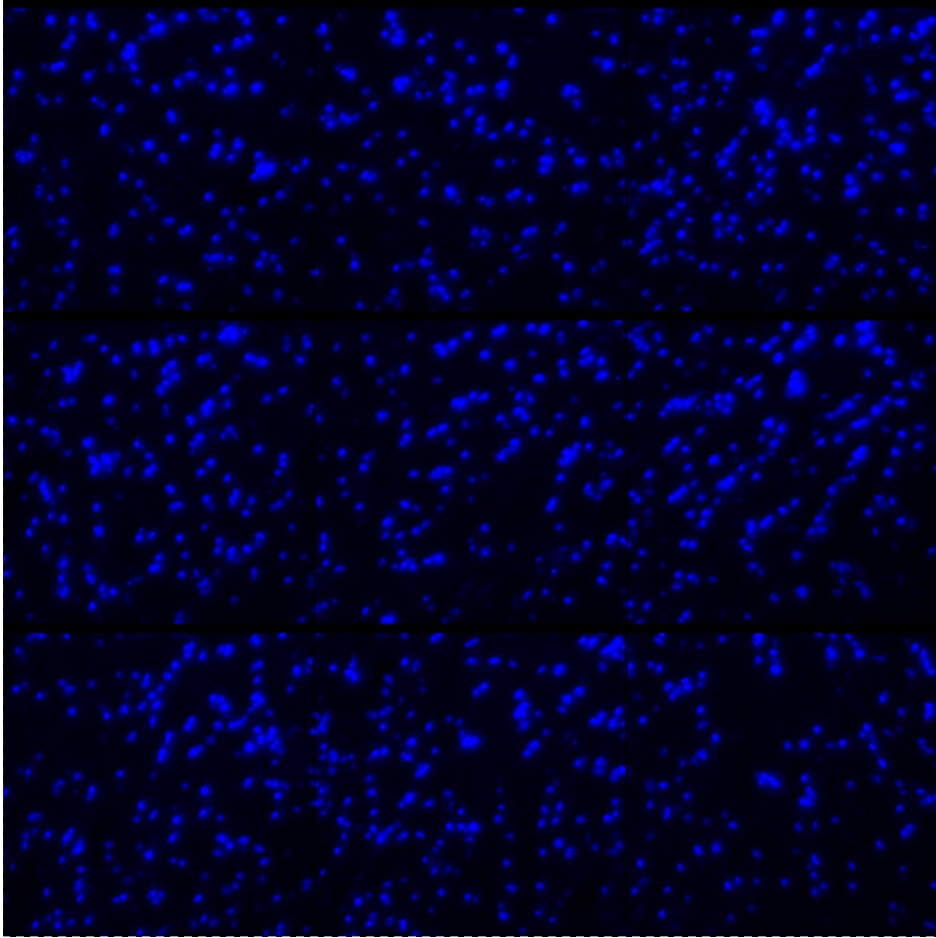


D2-6

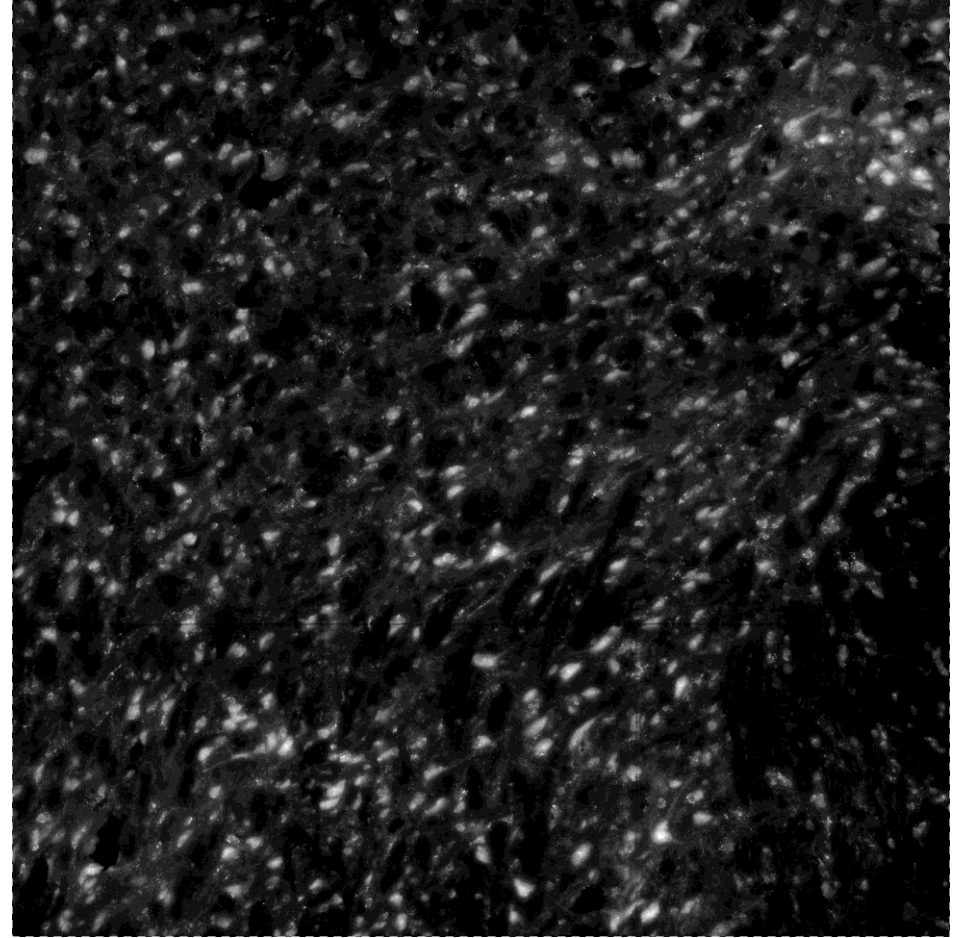


What we provide – Images

DAPI (maximum z-projection)

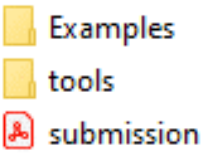
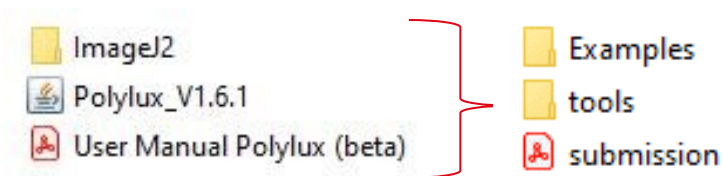


Raw (maximum z-projection of raw data from 1 channel)

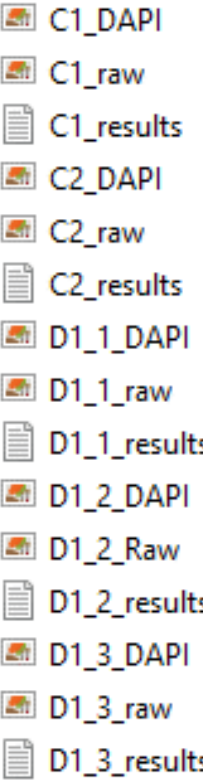


295.9 x 295.9 μm tiles
(2144 x 2144 pixels; 0.138 μm pixel size)

What we provide – Files



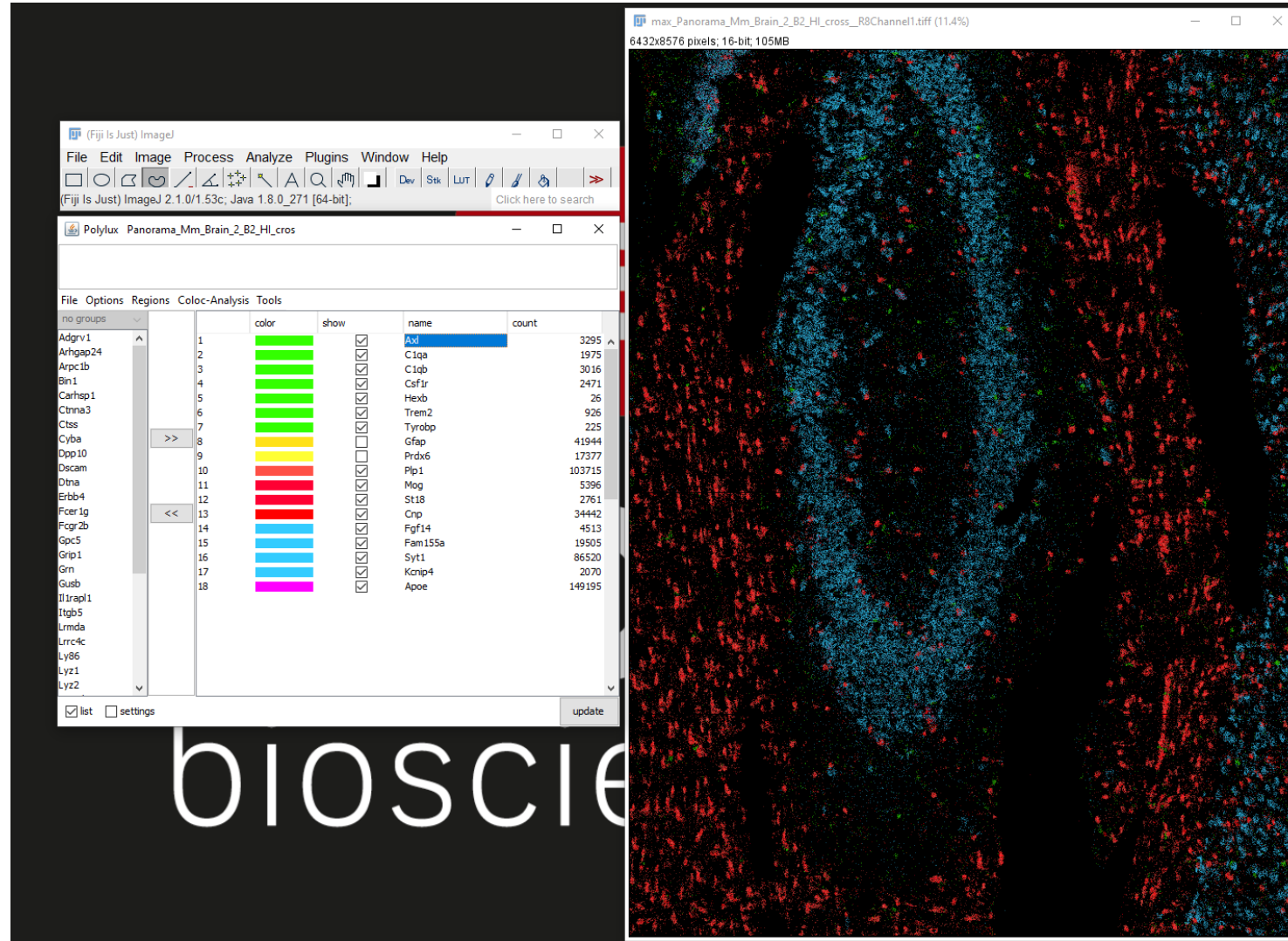
results files contain transcript coordinates
(in pixels, 300nm per z coordinate)



x	y	z	transcript
96	802	44	itga5
227	341	44	itga5
343	530	44	itga5
392	471	44	itga5
433	373	44	itga5
662	317	44	itga5
869	1250	44	itga5
1285	1674	44	itga5
101	910	15	cyp26b1
235	561	15	cyp26b1
387	1514	15	cyp26b1
650	296	15	cyp26b1
34	854	16	cyp26b1
40	844	16	cyp26b1
47	853	16	cyp26b1
49	872	16	cyp26b1
252	528	16	cyp26b1
258	549	16	cyp26b1
267	529	16	cyp26b1
272	542	16	cyp26b1

What we provide – the Polylux tool

- ImageJ plugin for easy visualization and data export.
- Requirements: up-to-date 64-bit Java and ImageJ2
- Tutorial video available here:
<https://storage.googleapis.com/apollo-customer-data/ServiceTransfer/PolyluxTutorial1.mp4>





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