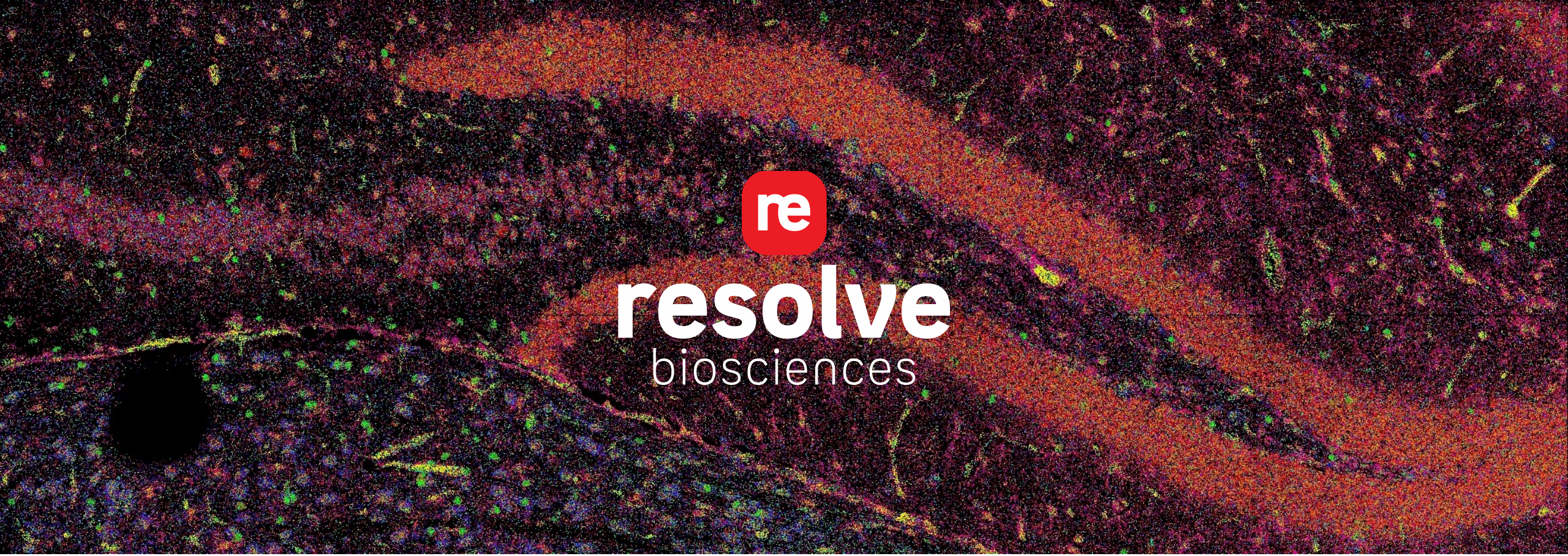


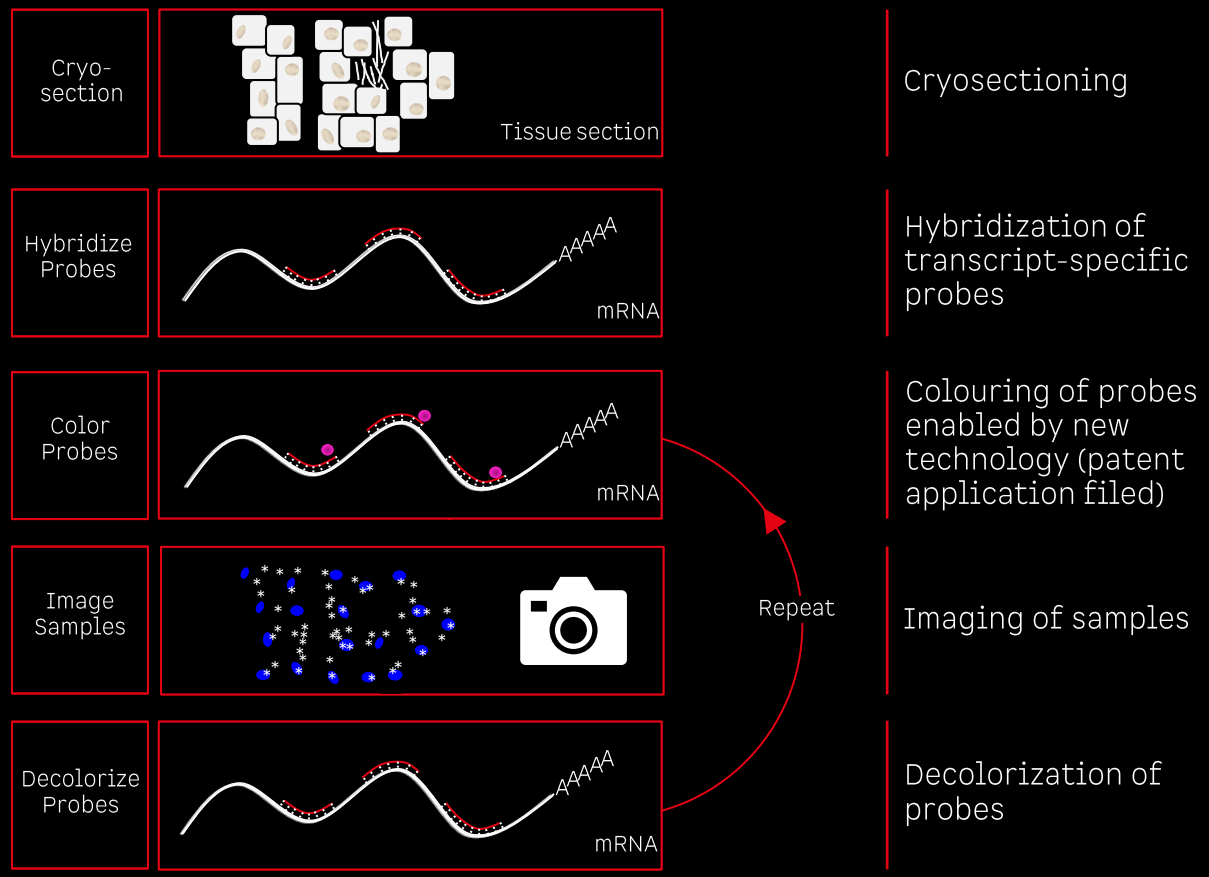
Every Cell Matters:
Molecular Cartography™ of the mammalian brain

Benedikt Nilges, Jeroen Aerts, Frank Reinecke, Annette Bogdoll, and Nachiket Kashikar

Resolve BioSciences GmbH

Molecular Cartography

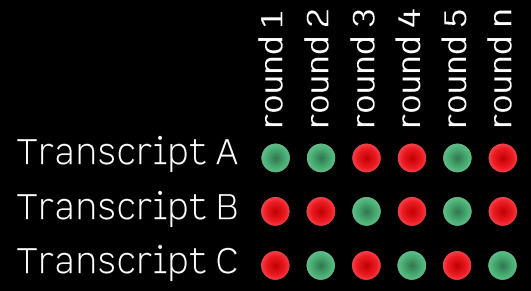
Combinatorial single molecule FISH



Key features:

- Up to 100 transripts in parallel
- Subcellular resolution
- High sensitivity
- Digital readout (transcript coordinates) for quantitative analysis
- Non-destructive: tissue remains available for follow-up experiments

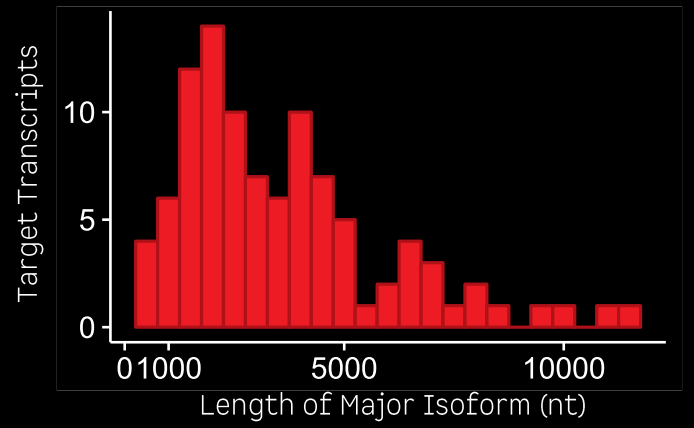
Code:

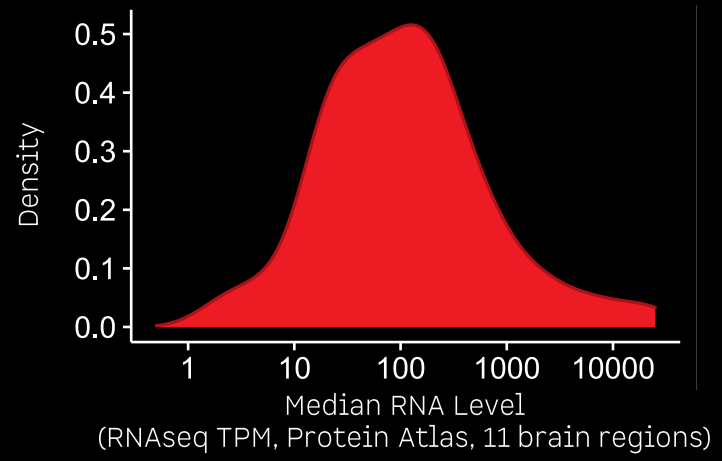


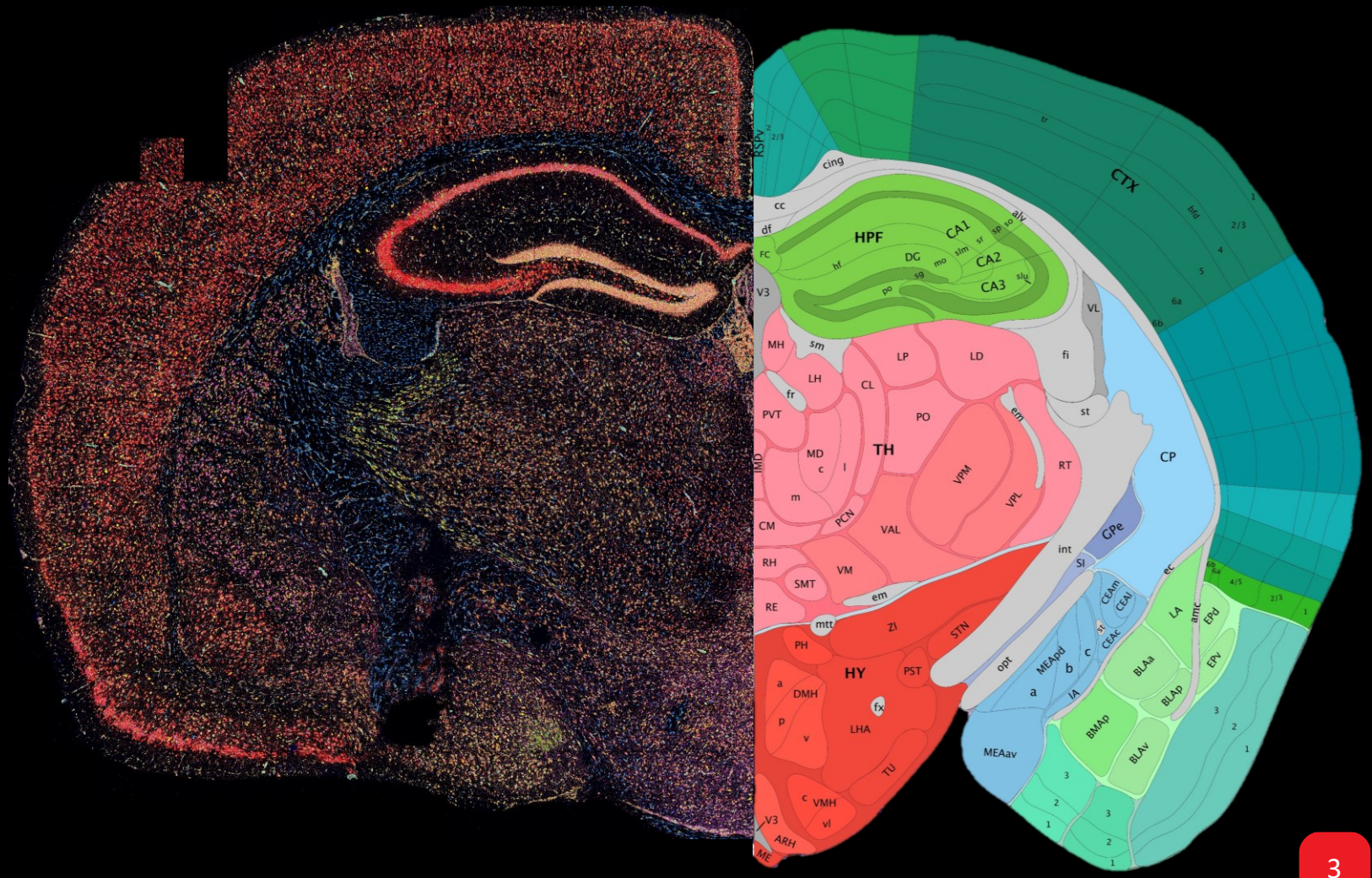
Molecular Cartography of Mouse Brain

100 marker gene panel
(balanced target length and expression)

~ 15×10^6 single molecule coordinates, 0.054% false positives
(randomized transcript colors)





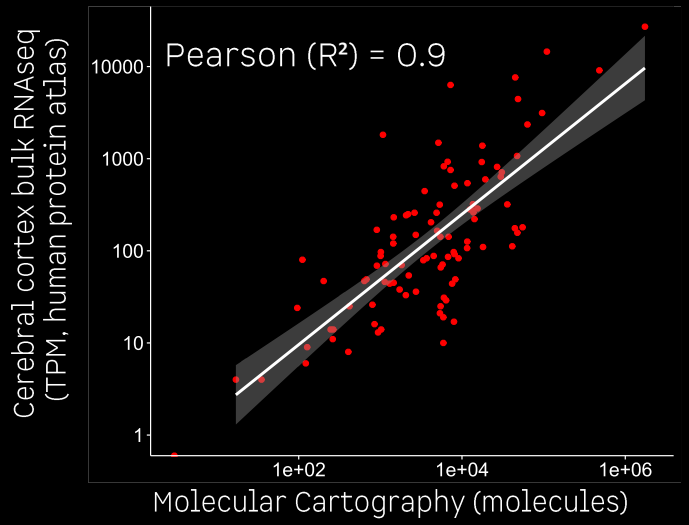


Mouse Reference Atlas – Allen Brain Atlas

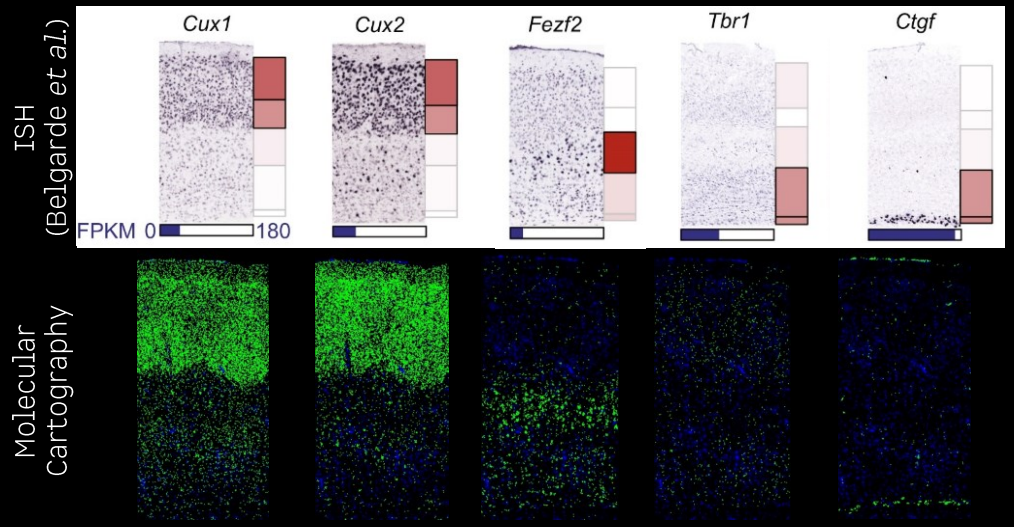
Quantitative and Specific

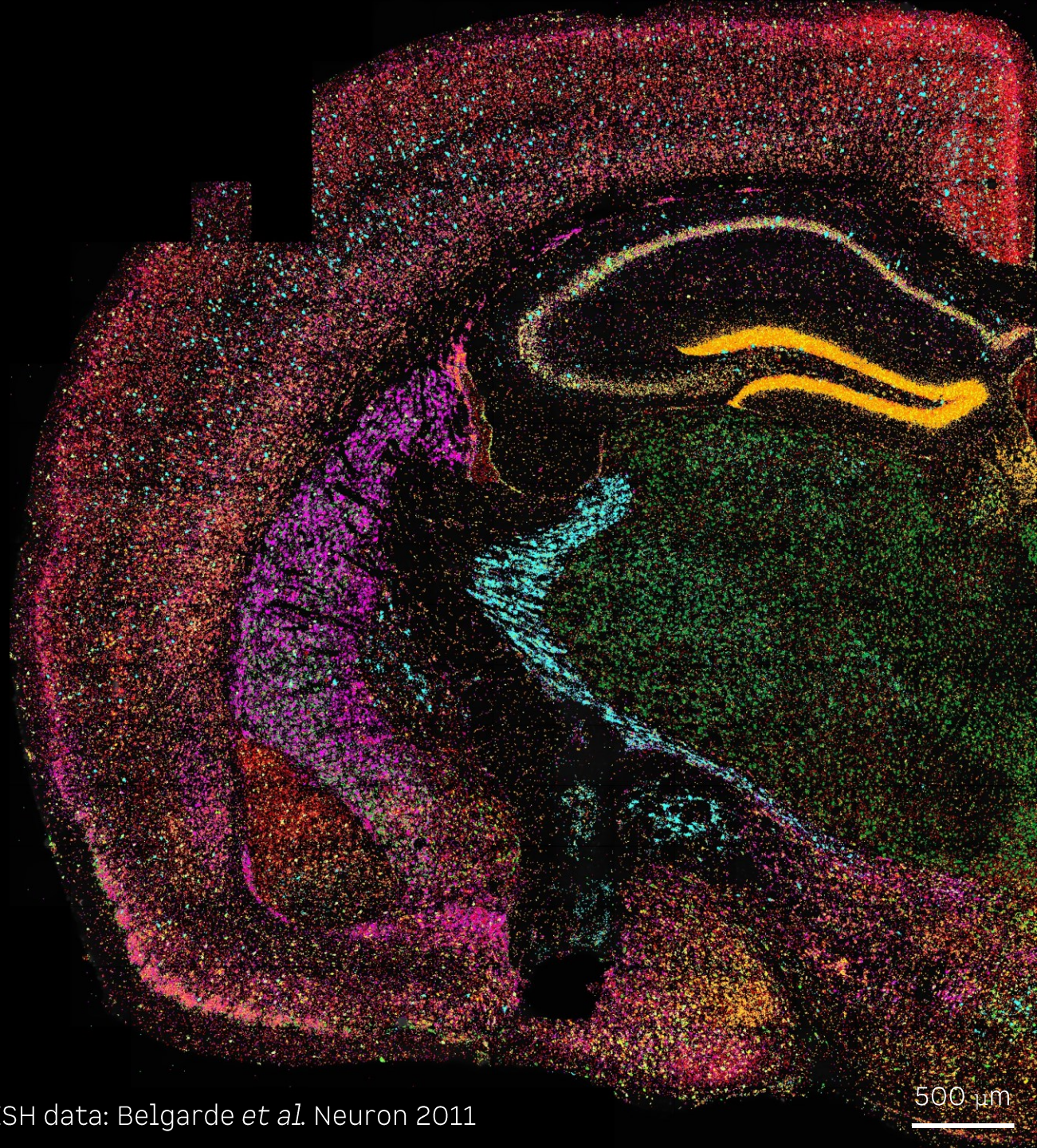
- Shox2
- Prox1
- Sema5a
- Bcl6
- Tabata
- Kit
- Cux2
- Tshz2
- Fezf2
- Tbr1
- Meis2
- Pvalb
- Penk

Cortical counts compared to unmatched RNAseq



Comparison to published cortical transcript distribution

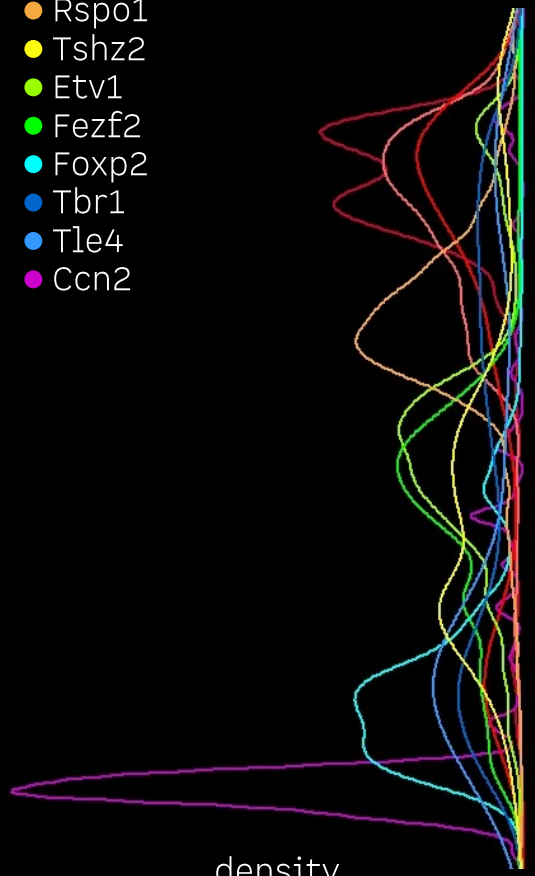


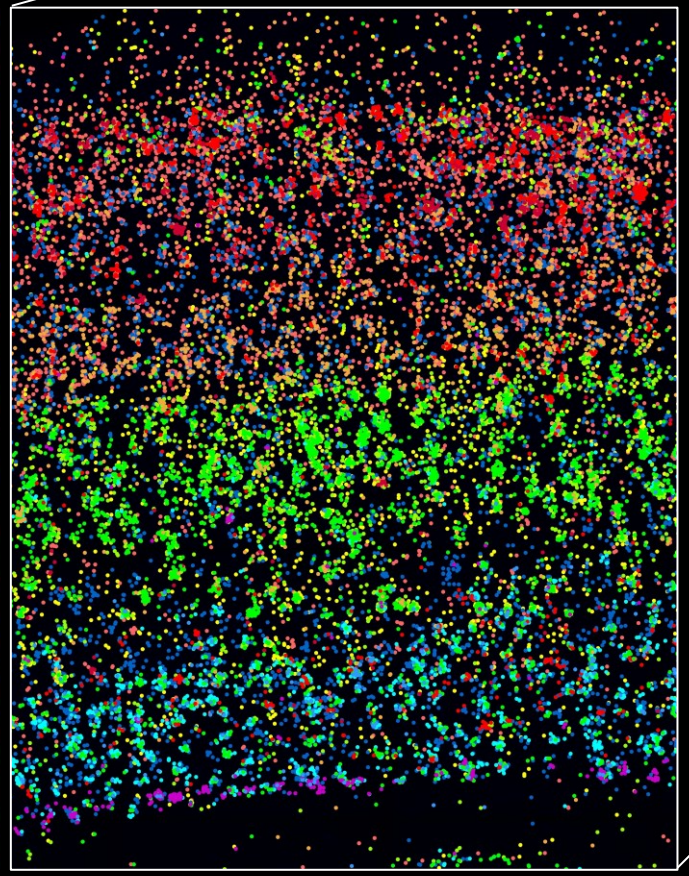


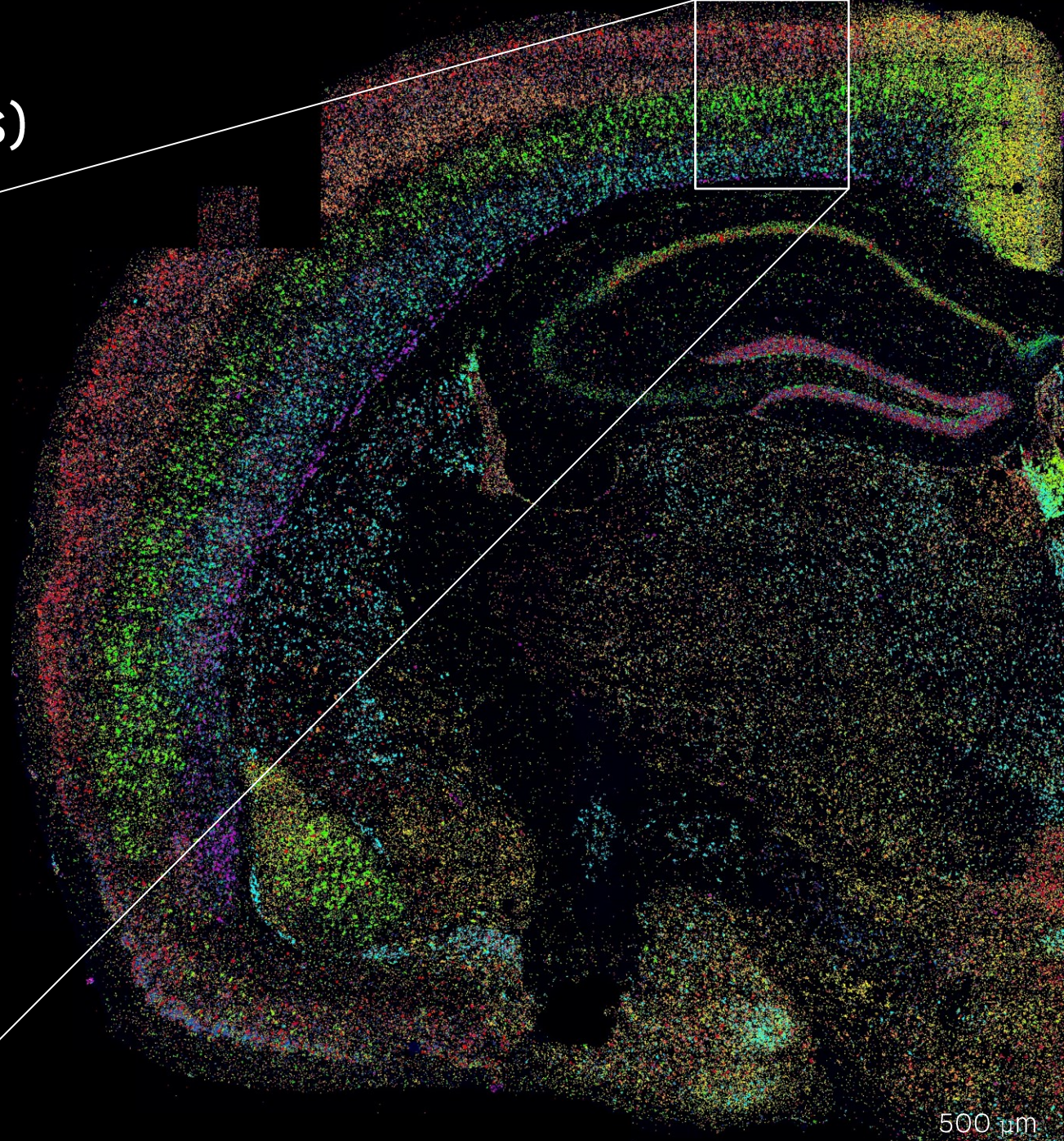
ISH data: Belgarde *et al.* Neuron 2011

Cortical Markers (11/100 genes)

- Calb1
- Egr2
- Cux2
- Rspo1
- Tshz2
- Etv1
- Fezf2
- Foxp2
- Tbr1
- Tle4
- Ccn2



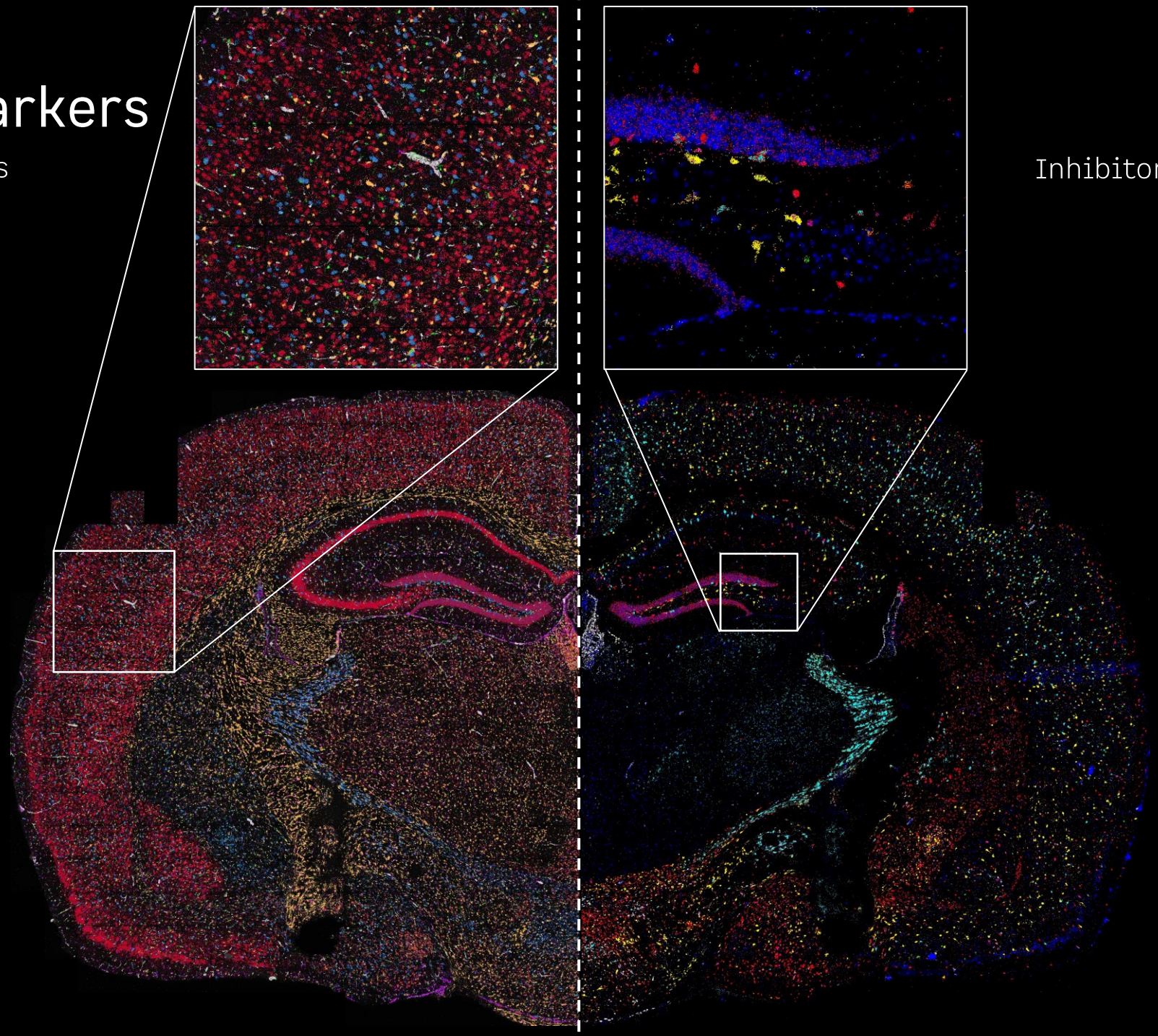




Cell-type Markers

Major cell-types

- Aqp4, Id3 — Astrocytes
- Olig2, Sox10, Plp1 — Oligodendrocytes
- C1qa, Csf1r, Spi1, P2ry12, Gpr34 — Microglia
- Chat — Cholinergic neurons
- Slc17a7, Cux2, Slc17a6, Rspo1, Fezf2, Foxp2 — Glutamatergic neurons
- Gad1, Sst, Pvalb, Th, Vip, Npas1, Htr3a, Chodl — GABAergic neurons
- Flt1, Pdgfra, Myh11, Pln — Other (SMCs, Pericytes VLMCs, Endoneurial cells)



Inhibitory neurons only

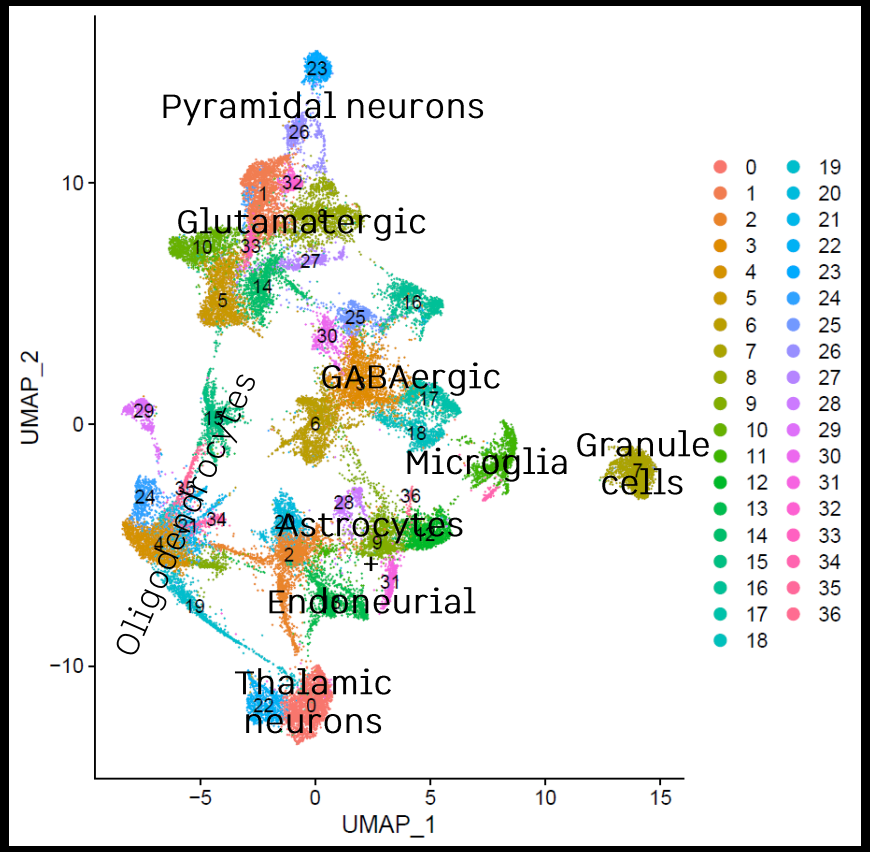
- DAPI
- Gad1
- Sst
- Pvalb
- Vip
- Lamp
- Sncg
- Npas1
- Htr3a
- Chodl

Cell Clustering (37954 DAPI-segmented cells)

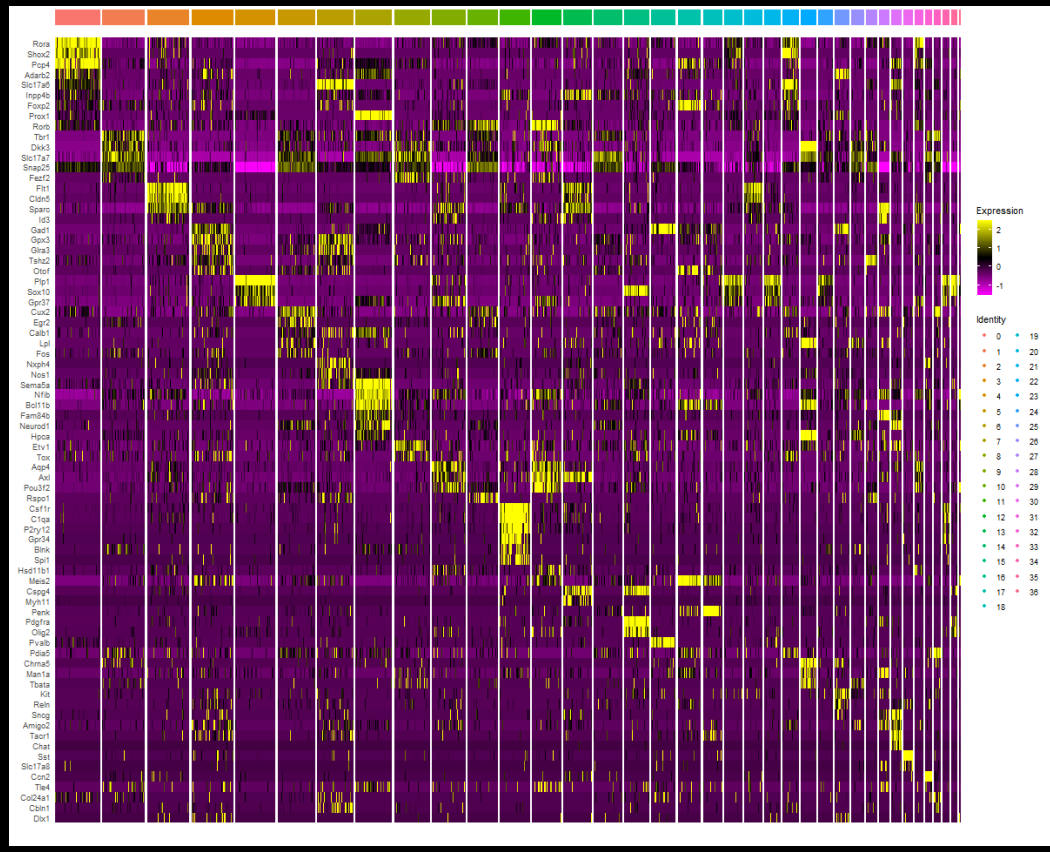
Signals per Cell

Median molecules: 251
Median genes: 24

UMAP plot based on cluster of segmented cells

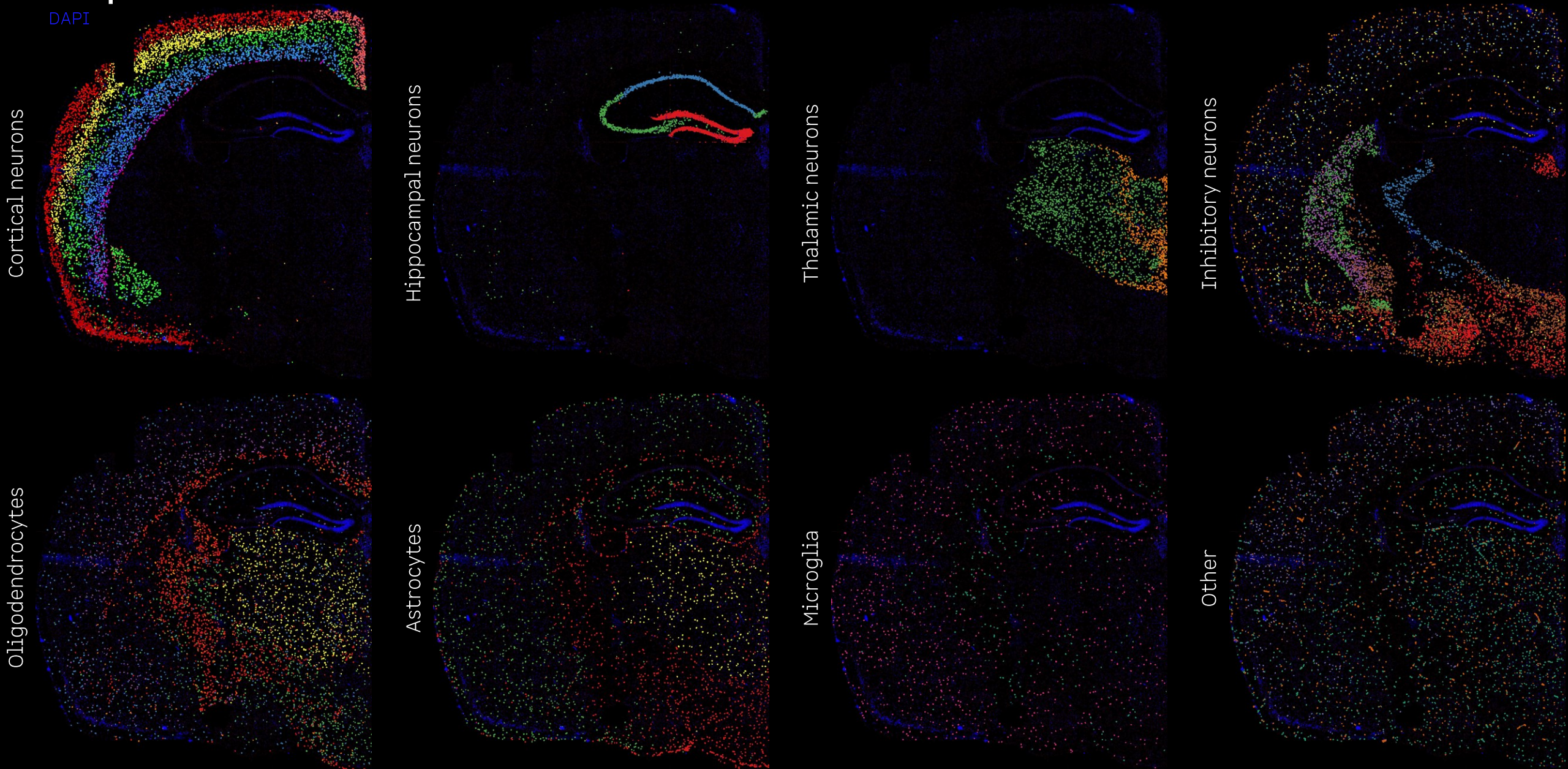


Heatmap matching UMAP plot
(log-normalized counts relative to all other cells)

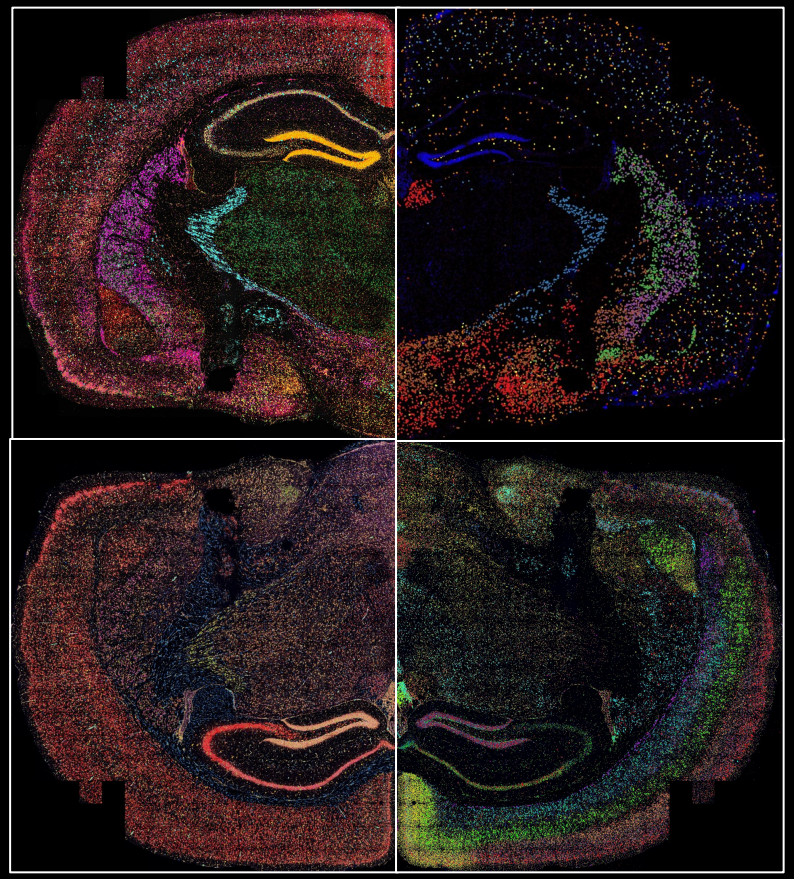


DAPI segmentation and 8 µm expansion via QuPath: Bankhead *et al.* Scientific Reports 2017
Clustering via Seurat: Stuart, Buttler *et al.* Cell 2019

Spatial Distribution of Cell Clusters



Summary



- MC is specific: matches conventional *in situ* hybridization
- MC is highly quantitative: correlation with RNAseq across wide range of expression levels
- Identification of cell-types enabeled by high sensitivity (>30 subtypes)
- Identification of all major cell layers and the heterogenous cell populations therein (scRNAseq-like analysis with spatial information)

- Resolve is making a multi-replicate data package available to the community.

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