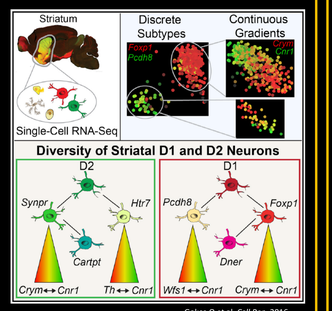
Incorporation of spatial mapping into single cell RNA sequencing workflows using a multiplex in situ hybridization technology

Jyoti Phatak, Han Lu, Li Wang, Hailing Zong, Bingqing Zhang, Xiao-Jun Ma
Advanced Cell Diagnostics, A Bio-Techne Brand, 7707 Gateway Blvd., Newark, CA 94560, USA

Introduction

Complex and highly heterogenous tissues such as the brain are comprised of multiple cell types and states with exquisite spatial organization. Single-cell RNA sequencing (scRNA-seq) is now being widely used as a universal tool for classifying and characterizing known and novel cell populations within these heterogenous tissues, ushering in a new era of single cell biology. However, the use of scRNA-seq presents some limitations due to the use of dissociated cells which results in the loss of spatial context of the cell populations being analyzed. Incorporating a multiplexed spatial approach that can interrogate gene expression with single cell resolution in the tissue context is a powerful addition to the scRNA-seq workflow. In this study, we used the RNAscope Multiplex Fluorescent and RNAscope HiPlex *in situ* hybridization (ISH) assays to confirm and spatially map the diverse striatal neurons that have been previously identified by scRNA-seq in the mouse brain (Gokce *et al*, *Cell Rep*, 16(4):1126-1137, 2016). We confirmed the gene signatures of two discrete D1 and D2 subtypes of medium spiny neurons (MSN): *Drd1a/Foxp1, Drd1a/Pcdh8, Drd2/Htr7*, and *Drd2/Synpr*. The heterogenous MSN subpopulations were marked by a transcriptional gradient, which we could spatially resolve with RNA ISH. Numerous striatal non-neuronal cell populations identified by scRNA-seq, including vascular cells, immune cells, and oligodendrocytes, were also confirmed with the multiplex ISH assay. Finally, the spatial relationship between the D1 and D2 MSN subtypes identified by Gokce *et al.* was visualized using the RNAscope HiPlex assay, which allows for detection of up to 12 RNA targets simultaneously in intact tissues. In conclusion, we have demonstrated the utility of two multiplexed RNAscope ISH assays for the confirmation and spatial mapping of scRNA-seq transcriptomic results in the highly complex and heterogenous mouse striatum at the single cell level. Incorporating spatial mapping by the RNAscope technology into single cell transcriptomic workflows complements scRNA-seq results and provides additional biological insights into the cellular organization and functional states of diverse cell types in healthy and disease tissues.



Gokce O et al, *Cell Rep*, 2016

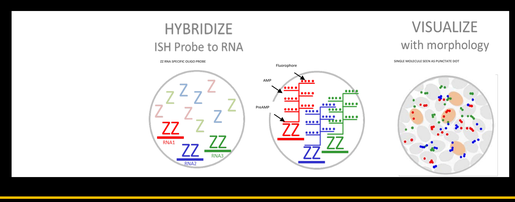
RNAscope Technology and Experimental Design

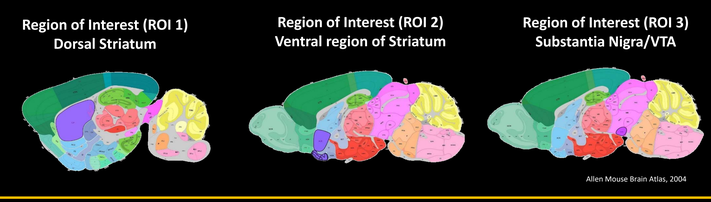
Tissue preparation: Sagittal sections (10 µm thick) of fresh frozen brain tissue from 6 week old C57/BL6 male mice were purchased from Acepix.

RNAscope™ *in situ* hybridization: The RNAscope Multiplex Fluorescent Assay and the RNAscope HiPlex Assay (8 reagent kit and HiPlex 12 Ancillary upgrade kit) from Advanced Cell Diagnostics were used for gene expression analysis in the brain, with a focus on the striatum.

Imaging and quantification: Images were acquired using the Zeiss Axio Z1 fluorescent slide scanner microscope with the Zeiss Zen2 image analysis software. Composite images were generated using RNAscope HiPlex image Registration Software.

Figure 1. Assay workflow and brain regions analyzed in this study. (A) The RNAscope Assay workflow. (B) Regions of the brain selected for analysis in this study.



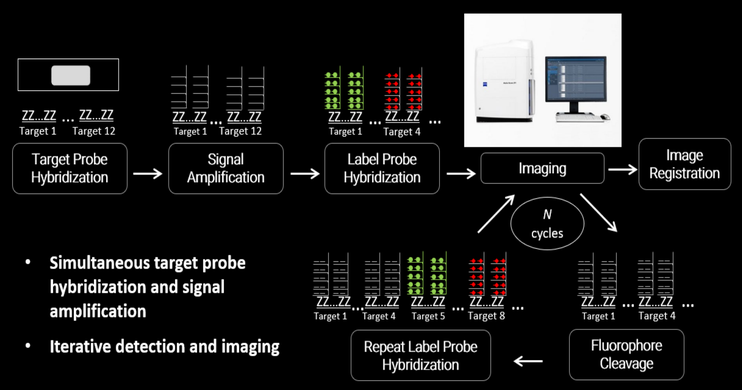


Allen Mouse Brain Atlas, 2004

RNAscope HiPlex Assay

Results

Figure 4. *In situ* hybridization workflow using the RNAscope HiPlex assay and the RNAscope HiPlex image registration software.



- **Simultaneous target probe hybridization and signal amplification**
- **Iterative detection and imaging**

Figure 5. Spatial mapping of all the *Drd1a/Drd2* striatal sub-populations in mouse brain. 12 target probes were used to simultaneously detect *Drd1a, Drd2, Foxp1, Pcdh8, Synpr, Htr7, Meis2, Calb1, Crym, Cnr1, Wfs1,* and *Th* using the HiPlex 12 Ancillary reagent kit

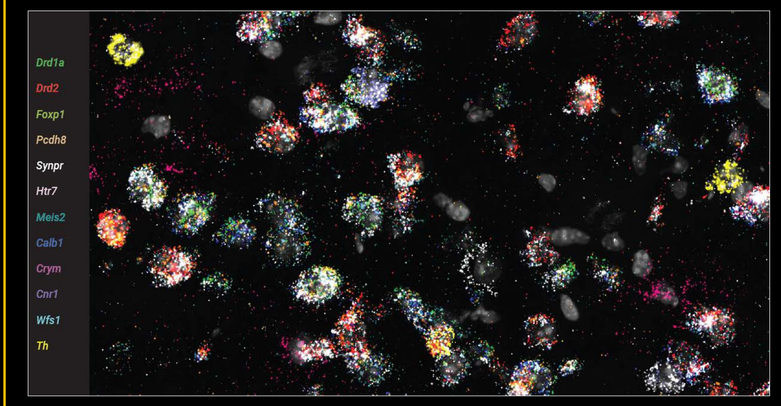


Figure 6. Spatial mapping of *Drd1a* major and minor sub-populations in the mouse brain. 8 target probes were used to simultaneously detect *Drd1a, Foxp1, Meis2, Dner, Cnr1, Crym,* and *Wfs1*

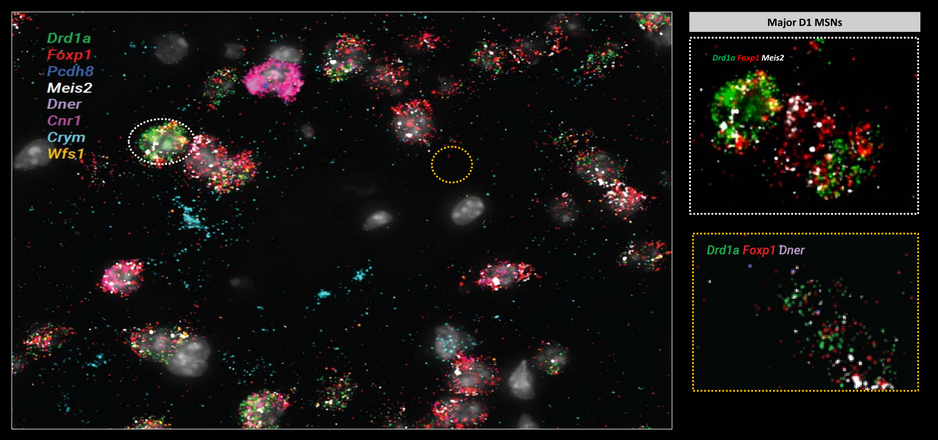


Figure 7. Spatial mapping of *Drd2* major and minor sub-populations in the mouse brain. 8 target probes were used to simultaneously detect *Drd2, Calb1, Cartpt, Synpr, Htr7, Crym, Cnr1* and *Th*

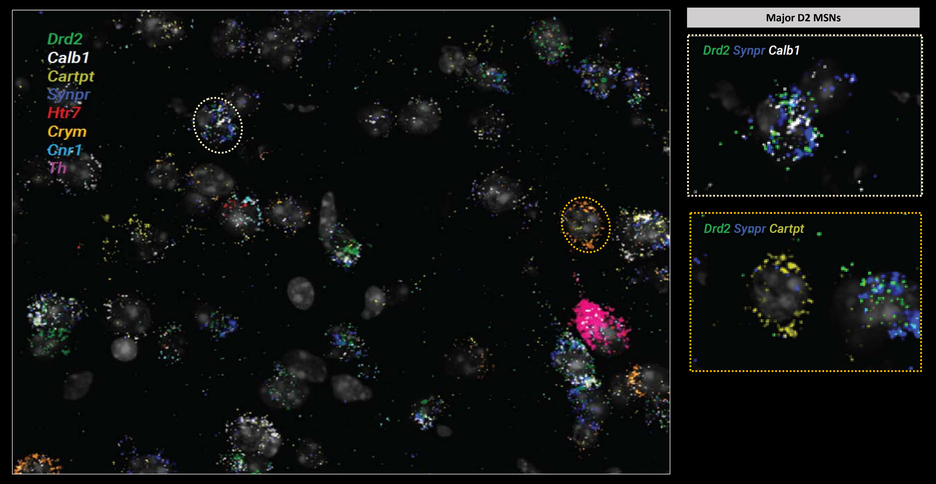
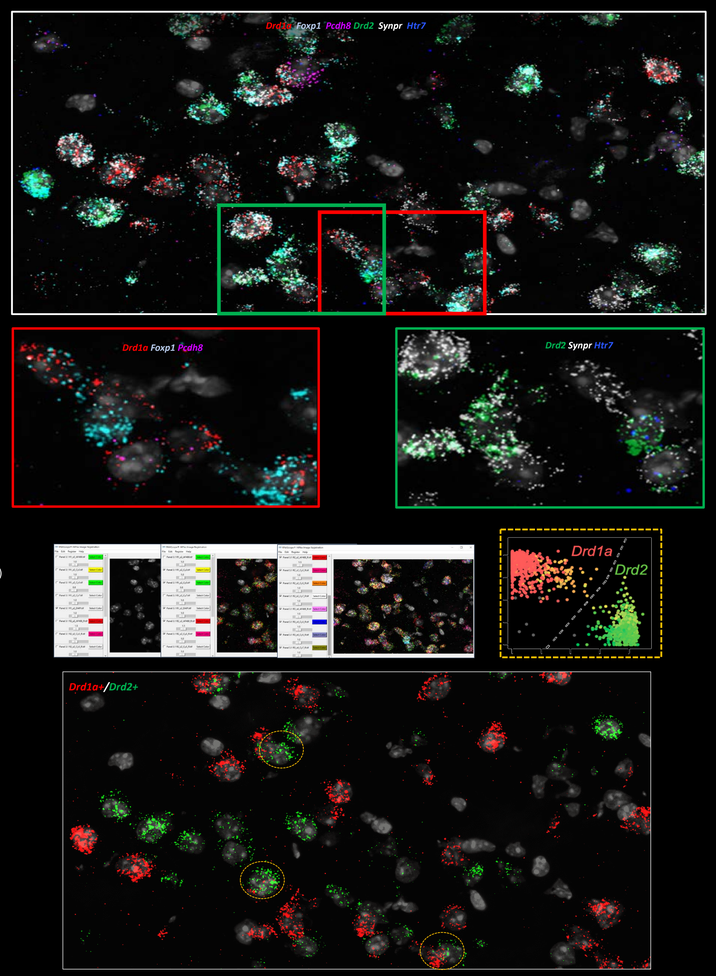


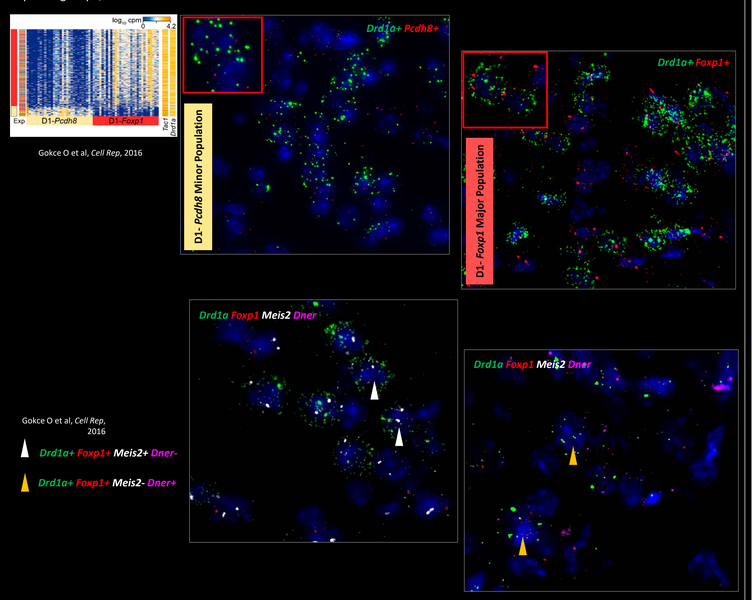
Figure 8. Detection of the major and minor D1 and D2 subtypes simultaneously in the mouse brain. (A) Visualization of the D1 (Red) and D2 (Green) major and minor populations on the same sagittal section. (B) Detection of cells co-expressing *Drd1a* and *Drd2* (marked by yellow circles), which could be done in the same section/field of view as in (A) using the HiPlex Image registration software.



Striatal Medium Spiny Neuronal Sub-Types

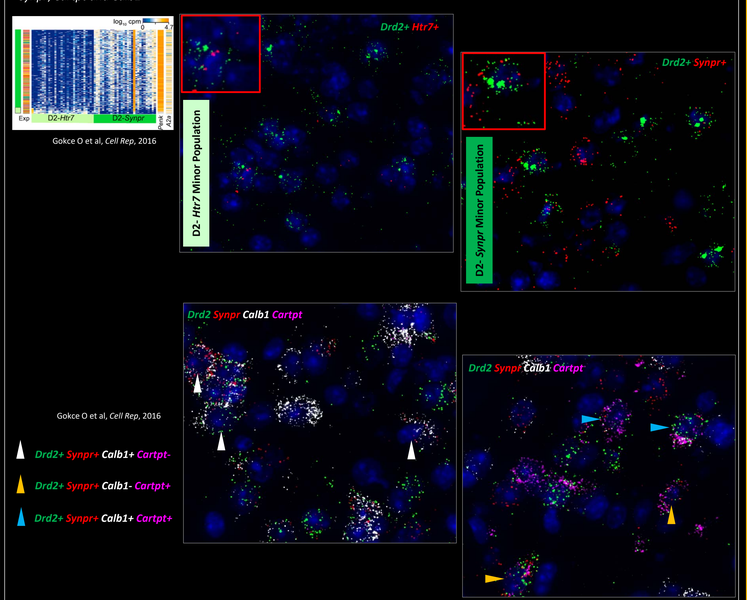
Drd1a Medium Spiny Neurons

Figure 2. Spatial resolution of the *Drd1a* cell types. (Upper) Major/Minor *Drd1a* sub-populations; (Lower) *Drd1a* major sub-type expressing *Foxp1, Dner and Meis2*

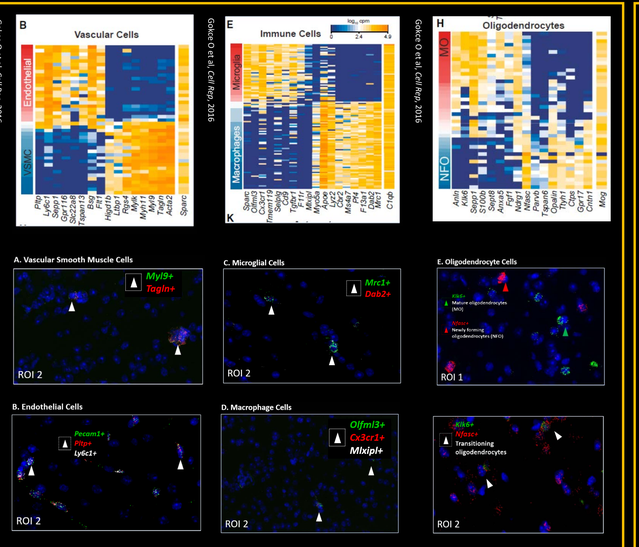


Drd2 Medium Spiny Neurons

Figure 3. Spatial resolution of the *Drd2* cell types. (Upper) Major/Minor *Drd2* sub-population; (Lower) *Drd2* major sub-type expressing *Synpr, Cartpt* and *Calb1*



Non-Neuronal Striatal Cell Types



Conclusion

Complex tissues with high cellular heterogeneity require single cell technologies both at the transcriptomic and spatial level to fully interrogate these cell types. In this report we have demonstrated the capabilities of a multiplexed in situ transcriptomic approach for the confirmation and spatial mapping of scRNA-seq results in the highly complex and heterogenous mouse striatum using both the RNAscope Multiplex Fluorescent and HiPlex assays. Numerous researchers from around the world have utilized the RNAscope technology to incorporate spatial analyses into their scRNA-seq workflows and further single cell research in neuroscience and beyond. Single cell transcriptomics combined with spatial mapping by RNA ISH holds great promise in resolving heterogeneous tissues at cellular resolution and providing insights into cellular organization and function of diverse cell types in healthy and disease states.

References

- Gokce O, Stanley GM, Treutlein B, Neff NF, Camp JG, Malenka RC, Rothwell PE, Fuccillo MV, Sudhof TC, Quake SR. Cellular Taxonomy of the Mouse Striatum as Revealed by Single-Cell RNA-Seq. *Cell Rep*. 2016;16(4):1126-1137.
- Allen Mouse Brain Atlas, 2004.